at

ATDEPARTMENT OF HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION

CENTER FOR DEVICES AND RADIOLOGIC HEALTH

EFFECTS OF RADIOFREQUENCY ENERGY (RF) EXPOSURE ON MICRONUCLEUS FORMATION

VOLUME I

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C O N T E N T S

P R O C E E D I N G S

DR. OWEN: We will just go ahead. When speaking, since we have a transcriptionist, please try to speak into a microphone. Also, if anybody hasn't signed in, there is a sign-in sheet just outside the door and please do so.

To start off today, I would like to introduce Lillian Gill. She is the Center for Devices and Radiological Health Deputy Director for Science, and she will be giving you welcoming remarks.

Welcome

DR. GILL: Good morning to all of you, and it is nice to see you here this morning. I am the Acting Deputy Director for Science in the Center for Devices and Radiological Health. As most of you may know, Dr. Elizabeth Jacobson was in the position before and she is now Acting Director for Science in the Commissioner's Office. So, I am standing in for Liz while she is away on detail, and I followed Liz in the Office of Science and Technology and, as luck would have it, this is an issue that I was beginning to become involved in, in '94, when I did leave the Office of Science and Technology to go to the Office of Compliance. So, I have been away from it for a while and will be interested to hear what progress has been made today.

I am also happy to be here on behalf of the Center Director, Dr. David Feigal, to welcome you to what promises

to be a very productive meeting. Today we are going to be exploring future directions for research on the effect of radiofrequency exposure on micronucleus formation.

I would like to acknowledge the many of you who have made a great effort to be here today. It is exciting to look around both tables as well as the back of the room, those behind me, to see so many of you from the scientific community here to share a common goal with us, to ensure that future research helps to answer the scientific questions about the biological effects of radiofrequency from mobile phones.

The Cooperative Research and Development Agreement, or the CRADA as we tend to call it, that the agency signed in June with the Cellular Communications Industry Association, is an exciting step in working with industry to find some of those answers. We hope that the work of this group will fill the knowledge gaps that have been raised by studies conducted to date.

We are looking forward to the role that the FDA will play in helping to explore the direction of future research. Again, I extend to you a very warm welcome for being here today, and I am sure you will have a very productive meeting. I would like to turn it back over to Dr. Owen.

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DR. OWEN: Thank you very much. Since I am giving only very brief comments and not using any audiovisuals, I think I will just speak from the table, here. In case anybody missed it, I am Russell Owen. I am Chief of the Radiation Biological Branch of the Center.

I would like to thank you for your interest and your attention to this meeting, and I wanted to point out that the goal of this meeting is to review the research on the effects of RF exposure to micronucleus formation. It is a very technical meeting, with a very narrow focus. Because of that and the importance of the issues that are related to it, we have tried to assemble the best group we can of experts in the topic area to get input on the kind of research that is needed in this particular area.

I should point out that the people that we have assembled here are assembled to represent their own expertise and are not here to represent any particular agencies or institutions.

As Lillian mentioned, the FDA has quite a bit of history in rad health, and has a mandate here that a lot of people don't know about because Food and Drugs doesn't say anything about radiation. In addition to our own research, we try and coordinate with several other federal agencies, both informally and through committees, and to participate in programs such as the World Health Organization

International EMF Project. All this is with an eye to getting the best scientific information assembled to assess the possible impacts of exposures.

As also described, another part of these activities in this area is the Cooperative Research Agreement with CTIA, and this meeting is a part of that project. CTIA has committed to contract for research consistent with the recommendations that we developed with the input that we gather at this meeting.

So, finally, I just want to point out again that the emphasis here is the science because it is the foundation of all the assessments that we do and also the common literature base or database for all expert groups that assemble to make judgments on possible health effects of various exposures.

I am pleased now to introduce Dr. Greg Lotz as our first speaker. Dr. Lotz is Chief of the Non-Ionizing Radiation Section, Division of Applied Research and Technology at the National Institute for Occupational Safety and Health. I will only say that once; it is NIOSH. Dr. Lotz will be giving us a broad introduction and background, and I think will give you a real good sense of the context that the information that we are going to collect here fits into, and also give you some feel for the background. Thank you, Greg.

Introduction and Background

DR. LOTZ: Good morning.

[Slide]

As Dr. Owen has indicated, my intent here this morning is to present a broad general background of where we are in bioeffects for radiofrequency exposure. We will hear the details about the specific area of micronuclei later and I will not attempt to address those. We are going to hear more about dosimetry from Howard Bassen, and Howard asked me a moment ago if I was going to talk about electric and magnetic fields and I actually had not planned to do so. So, I hope we will be able to interface that as well.

[Slide]

My intent is to do a couple of things, knowing that we have various backgrounds, some more familiar with RF literature than others and knowing that those who are quite familiar with it, of which we have many in the room, already know probably everything that I am going to say. So, I want to make a few comments about outlining the background of where we are with existing RF research; evaluate the general need for additional RF research; and present a rationale for why we would be considering research on micronuclei formation in particular -- as Russ mentioned, a very narrow focus for our meeting this week; and then talk about what I would consider to be key characteristics that we need to

consider in developing our product of this meeting, namely, the design for future research on micronuclei formation.

[Slide]

In the RF literature background, most of it has historically been oriented towards stronger exposures for longer periods of time in that it served to address concerns for what were primarily occupational groups exposed to radiofrequency radiation, such as military personnel exposed to radar or other personnel, other types of radar uses as well; many industrial uses and the groups of workers around those using heaters or sealers; and it would also include medical technicians and practitioners who use radiofrequency for various medical purposes such as diathermy.

In that, most of the literature is related to laboratory work with animals or in vitro preparations, and there is a general evidence in that literature of demonstrated effects at levels associated with tissue heating. One of the terms I think we will end up throwing around a lot this week is specific absorption rate, or SAR, the accepted unit of measuring, or estimating in some cases, absorbed energy from a radiofrequency exposure.

I wanted to just make a few comments here. There are some numbers that come into play here. Historically, the animal work in dealing with whole body exposures demonstrated that with exposures in the vicinity of 3-4 W/kg

you could elevate the temperature of laboratory animals exposed over rather short periods of time, a matter of minutes to a few hours. With that kind of exposure, particularly under certain frequency conditions, you could generate a significant amount of body heating and, indeed, even at exposures a little bit lower, in the neighborhood of maybe 1-2 W/kg, you can actually demonstrate that an animal's thermoregulatory responses, such as a change in metabolic rate or vasodilation, will actually be stimulated, clearly indicating that you have tissue heating going on in those circumstances.

That is for whole body exposure. As I will comment more in a moment, if you reduce that exposure to part of the body it changes that picture, as you might expect from a tissue heating standpoint and you don't see those same responses. But, in fact, that 4 W/kg many years ago, in the '80s, was selected, in the case of the IEEE ANSI guidelines in this country and ICNIRP guidelines, as the presumed threshold of bioeffects for whole body exposure.

One of the other numbers that will come into play a lot this week is 1.6 W/kg, and I won't go into all the details, but that is the local limit in this country. It basically comes from the IEEE guidelines and it is the local limit defined by the Federal Communications Commission for the SAR for a local tissue deposition averaged over one gram

of tissue. Now, that is a very different phenomenon than the animal being exposed whole body to an average of 4 W/kg. But those are some numbers to give a little context to numbers that I think we will be throwing around later with respect to dose.

[Slide]

Now, I might also comment there that when you move into the in vitro situation SAR is much more difficult to put into context because you have other parameters in the experiment. Usually there is an effort to prevent heat from building up within the system. So, it has a different significance that we will probably talk about as we get into individual experiments.

Also out of this body of literature, which has been generated over several decades, there was a conclusion that RF was not genotoxic. Now, additional aspects in the general RF background within the last ten years, within the '90s, are that we have new development in what I would call the nature of the RD exposure in terms of who is being exposed.

We now have the aspect of large populations being exposed repetitively or continuously to low levels of RF, in contrast to the stronger occupational exposures I mentioned a moment ago, and we are moving to a situation where potentially everyone will be exposed, or nearly everyone

will be exposed because of the use of wireless communications technologies. And, we are also here to talk particularly about a source, the cellular phone, that primarily generates a localized exposure, often of the head, of great concern in terms of the tissue exposed being the brain.

We have, in the context of that then, large populations being exposed for long periods of time, a lack of research in the literature on long-term effects of RF, either of humans or long-term animal studies. And, this is in sharp contrast to the situation with electric power frequencies or extremely low frequency ELF frequency exposures which have been very much on the minds of researchers in this arena in the last decade or so where there were many dozens of epidemiologic studies, some very sophisticated which were in many respects driving the whole issue and the interpretation of the literature. We do not have a body of literature like that in dealing with radiofrequency exposures.

Now, there are some studies under way and they are not really a topic of our meeting this week, but in terms of what is actually finished and reported there are very few long-term studies.

[Slide]

There have been three or really two panels convened around the work in the last couple of years. These are not the only reviews of the radiofrequency literature, but the Royal Society of Canada convened a panel which issued a report just a little over a year ago, in May of 1999; and the United Kingdom convened a panel, chaired by Dr. Stewart, sometimes referred to as the Stewart Panel, which just reported a couple of months ago having looked at the literature with particular interest in the question of wireless communications. Russ mentioned in his remarks the World Health Organization International EMF Project, and they have had workshops and reviews also to look at this literature.

[Slide]

So, what is good is that these panels have come up with similar findings, and these are just a few of those. This is not meant to be a comprehensive list. I want to start with the point that these panels have operated from an outlook at the start that interpretations of scientific knowledge should be based on work published in peer-reviewed journals. So, in come cases today and the next couple of days we are going to be talking even about work that is not yet published but dealing with micronuclei because of its interest, but their conclusions and their considerations were strictly based on the peer-reviewed published work.

As I mentioned a moment ago, certainly both these panels conclude that thermal effects -- from what I have phrased here just not to be too wordy -- is sufficient RF exposure to have potentially adverse health effects. By sufficient, I mean those that create a thermal load, in association in animals with other bioeffects such as behavioral changes or physiologic changes, hormone changes, things like that that were deemed either to be significant in terms of potential immediate health effects or to have ramifications that they would be effects that we would want to protect against. That is not to say that those effects, say at 4 W/kg, were necessarily immediately critical or anything like that.

I want to also say here, at this point, that the wireless communications sources that we are concerned with do not normally produce RF exposures of the magnitude related to these thermal effects that you find in the discussions of these panels or in the literature in general.

Both of these panels have concluded that there is evidence of biological effects at levels that do not cause measurable heating. Now, that level that does not cause measurable heating can vary depending on the conditions of the experiment and the subject, but some of those findings are generally related to <u>in vitro</u> effects. One that has received a lot of mention would be the activation of

ornithine decarboxylase, but there are others as well that have been cited by both these panels as evidence of effects at levels that do not cause measurable heating.

[Slide]

What are some of the other conclusions from these panels? That the existing evidence does not indicate that these low-level effects have adverse health consequences. In other words, the conclusions are there are reports in the literature of some effects. Those cannot be dismissed, but there is no indication that those effects would have adverse health consequences. Certainly there is no literature to connect them to adverse health consequences at this point.

But the panels have also concluded that the body of literature is inadequate to answer the questions we have about prolonged low-level exposure at this point.

Cancer is a dominant concern in terms of long-term low-level RF effects but there are other health concerns that exist, primarily neurological and more subtle, that are certainly unresolved at this point.

And, while many studies in the literature point to an absence of effects of concern, there are some key studies that exist that raise questions about the potential long-term low-level health effects, and that includes a few animal studies primarily although there are some human

studies but those have some flaws that limit their interpretation.

[Slide]

Why are we here to study micronuclei? We probably all have our own thoughts on that. But, basically, my interpretation of our objective for this week to look specifically at micronuclei is that we now have evidence of effects in more than one laboratory, and that data is going to be reviewed by others, much more qualified to do that than I, here today. The potential importance of any finding of effects involving DNA is a factor in why the micronuclei issue itself comes to the forefront.

And, I want us to think about the consideration of this effect not for any effort to say there might be a specific health effect as an outcome, and I don't think that is a supportable argument at this point, but as an indicator of plausibility. If, in fact, we find reason and future research supports the idea that there are effects on micronuclei then what does that tell us about the plausibility of RF effects related to DNA and potentially related to issues of long-term health consequences -- a question of plausibility, not tied to any particular health concern at this point.

Finally, I think one of the things we need to really consider carefully in this is what kind of

understanding can we reach of the meaning of micronuclei changes in the context of the generally negative genotoxicity results? We certainly have others here who can comment better on that than I, but that I think is one of our key questions.

[Slide]

Now, as we consider the design of further research on the formation of micronuclei -- I realized after I made these two slides that we have a situation where I have skipped over in the slides what might be the obvious things of what are the biological parameters. If we are doing in vitro studies, what are the best cell models to use? Does there need to be any work on in vitro exposures of which there have been a few reports in the literature? Cells from animals or humans exposed, and then looking at micronuclei formation in those cells. For in vitro studies how long should the cells be exposed? What are the proper assay conditions? All those kinds of things.

[Slide]

But in a more general sense I want us to also have a strong emphasis on these points that I have listed here, and that would include both the biological aspects of the study along with the dosimetry. We have a lot of concern about the dosimetry at this point because of the dosimetry of the devices that are creating this exposure of humans;

the complicated aspects of a major concern being local SAR, localized exposure of the human as opposed to whole body exposure. So, we have a lot of emphasis on the dosimetry and a lot of concern. That needs to be in there, but I am concerned that we not short-change the biological aspects of our qualifications and design on that.

What that basically speaks to is that the research team that is going to do the work that we are speaking to as we say what we think needs to be done, needs to be a multi-disciplinary team that can strongly address both these. We will not be well served if we have researchers who have great expertise in the biological aspects but little awareness and not enough interaction on the dosimetry side, or vice versa. So, I think that is a very important consideration as we go here.

I think we need to have very clear expectations of carefully defined protocols and the use of positive controls in the experiment. I know that the work that we are going to hear about today has already included those kinds of things, but as we talk about what the characteristics of future work are, some might say, well, those are obvious but I think in the radiofrequency literature you find that they are not obvious, and that is why I bring them to the forefront because we need to have those kinds of things up front in our experimental work in this topic.

We need to have aspects of the biological model that is being tested be carefully characterized, things like the inherent variation within the biological system and the assay that is used. Again, some may say, well, that is obvious but it hasn't been obvious in some of the work that has gone before, not necessarily specifically on micronuclei but others, and we need to keep that at the forefront.

[Slide]

One of the things we need is repeatability of findings in the laboratory. In the RF literature we will find studies reported where the experimenters have done it once basically and reported the finding. We need to have more repeatability and demonstration of that in the research that we are calling for in these new studies.

We need to have an evaluation of the dose response of the effects, if those effects are observable in the research, over a range of specific absorption rates, SARs. I want to make the point here that I think that needs to include SARs that might be considered thermogenic -- the dosimetry, the experimental model, how temperature is handled in the model if that is <u>in vitro</u>, all are factors in that. But even at the point of going to higher SARs than might be of interest in human exposure, we need to have these studies go into that level to have, if you will, a more traditional toxicological approach to demonstrate the

effect and then look for a threshold, if there is one, that considers a range of SARs. So, the dose-response range is very important to me as we go forward on this.

Then, from there, we can look at the evaluation of the mechanism of those RF effects if demonstrated and supported. By mechanism, it comes to mind for me that we are talking two sides of that coin. We are talking the biological -- if there is micronuclei formation and the research shows there are valid findings, is that some kind of direct effect on DNA? Probably not perhaps, but what are the characteristics of that? Is it involved in the processing of the DNA? How does that manifest itself? Can we understand that process? As well as the biophysical mechanism of is this related to a heating phenomenon? Is it related to field interaction? Some have suggested free radicals might be an issue.

So, I think as we get into a discussion of mechanism there is both the biological side of that and a biophysical side. Those are the characteristics that are most on my mind as we start this and I think can kind of guide what is really a very focused goal for the week. Thank you.

DR. OWEN: Thank you, Dr. Lotz, for that very useful and thorough introduction. Our next speaker is Howard Bassen. He is Chief of the Electrophysics Branch

here, and he will be talking about radiofrequency absorption and dose. As Dr. Lotz mentioned, this is a very difficult area of research that requires interdisciplinary work, and understanding of RF absorption and dose is a complicated task.

RF Absorption and Dose

MR. BASSEN: Thank you. I am going to address the engineering aspects of this program, and I look forward to learning a little more about the bioeffects and health implications of this research. As was stated, I am the Chief of the Electrophysics Branch here, in the Office of Science and Technology at CDRH.

[Slide]

I would like to cover the dosimetry aspects, but first we need to cover a few of the very fundamental things. I am going to skip over some of the exposure parameters.

The Office of Science and Technology and the Electrophysics Branch has been involved with the development of implantable electric field probes and measurement instrumentation and computations. What you will see next is a discussion of some of the things that are involved in the dosimetry, mainly by the researchers who are involved in the field today as well as CDRH.

[Slide]

Dosimetry refers to the measurement or the computation of external fields and the induction of energy internal to a biological system. So, the dose inside an organism such as a cell culture or a human's head is produced by external fields from a cellular phone or a laboratory exposure system.

[Slide]

We will be talking about the fields and the SAR inside the biological tissue. SAR, or specific absorption rate, is measured in watts per kilogram, and it can only be produced in something that is electrically conducting such as water, biological material which is filled with water. So, the SAR in air is always zero because there is no electrical conduction in air. SAR is different at every single point in an object so that when we talk about SAR you have to be aware that from one point in the head when you are using a cell phone to a few centimeters away the SAR can be a hundred times different. The same thing would happen in your biological effect studies if you are not careful, and Dr. Chou will talk about those in the next speech.

[Slide]

The radiofrequency spectrum that we are talking about for cellular phones covers a frequency of either around 850 MHz, depending on the system, and I will talk about that later, or 1900 MHz for PCS phones, cellular or

PCS. This is the RF spectrum, from 3 MHz to 300 GHz, and 1 GHz is 1000 MHz. So, we are talking about frequencies in this range and their corresponding wavelengths, as you can see here, between 35 cm and at the higher frequencies 15 cm. So, outside the body these wavelengths are about the same size as the head. They couple well into the head because of that. But will they couple into a smaller biological tissue sample? That is the job of the in vitro dosimetry person.

[Slide]

Wavelength is a function of the speed of light and the frequency. I won't go into this in any depth, but if you would like the notes I would be glad to pass those out later.

[Slide]

Wireless handset frequencies, as you can see, in the U.S. used to be analog; North American digital; cellular GSM in Europe. These are all in the lower frequency band where the wavelength is about 30 cm. This should be 1850-1910 MHz. And, we are talking about a transmitter, the handset. So, these would be the frequencies. These are the base station frequencies that transmit back to the handset that would expose people at a distance even if they don't have a handset.

[Slide]

The important property of material that we are going to be interested is the conductivity. It is the measure of the resistance to electricity of the particular material and it is a function of frequency. The units of conductivity, and we will give that the symbol sigma, are in Siemens/meter or mhos/meter.

[Slide]

This slide shows the fact that every part of the body has a different value of conductivity. Muscle, bone and fat in the head or the in vitro sample will all have different conductivities. So, when we are measuring dose the external field is going to be absorbed differently depending on that plus the dielectric constant.

[Slide]

This slide shows the change versus frequency of conductivity versus frequency and dielectric constant. We are primarily concerned about this red curve. And, at the cellular phone frequencies we have something on the order of 1 S/m. At PCS it is a little bit higher. So, this sigma value will be important. This is for a particular type of tissue. This is probably muscle tissue. Bone would be a little lower. Brain tissue would be a little lower. So, we have to know sigma as well as measure the electric field in the tissue. I will go into that in the further discussion of dosimetry.

[Slide]

So, how do we measure SAR and what is SAR in terms of these parameters that I have been talking about? You can measure or compute the electric field inside a dielectric body. Remember, there is an outside electric or magnetic field from your cellular phone. However, we are talking about what gets inside the body, and only the electric field inside the body is important. Sigma, as we talked about it, is the conductivity. E^2 or the electric field squared in volts/meter is a function of SAR, and rho is the mass density of the tissue.

So, this shows you that SAR isn't really a basic parameter. It is a combination of sigma, the conductivity and electric field strength and the mass density. Why was SAR chosen in the first place as a measure of dose?

[Slide]

This slide will show you that. In the earlier days, when people were looking at thermal effects of microwaves, they measured the temperature or the thermal effects of microwaves at much higher powers than we get from a cellular phone and they simply measured the temperature rise over a period of seconds, and SAR is also equal to that. So, if you were worried about thermal effects you could measure the temperature rise in one or two seconds and determine the SAR that way. So, the important parameters

and specific absorption rate could be temperature rise, or electric field strength that we are actually measuring with our instruments.

[Slide]

Local and whole body SAR -- what do these terms mean? These are averaged over small gram of tissue or biological material. That would be the local specific absorption rate, watts/kilogram in one gram of tissue and this is measured by a small probe.

It varies, as I mentioned before, by a factor of hundreds of thousands from one side of the head to the toe if you are holding a cellular phone. The whole body average is a single value of what the whole person absorbs when using a cellular phone or standing near a base station or what an in vitro sample would absorb when you average all these variations, as I will show you in a later slide. So the local SAR is always going to be higher than the whole body average SAR, and you will see a better indication of that in a future slide.

[Slide]

As I mentioned, SAR is non-uniform always and you can get from 100-1000 times variation from one point to another and from local to whole body average SAR. So, the safety standards are based on local SARs and whole body average SARs. It gets rather complicated. For this work, I

believe most of the dose we will be talking about is local SAR.

[Slide]

Why is the SAR different in every part of the body or the sample? Well, this is an old cellular phone or it could be a dipolar antenna. We see outside the body or the in vitro tissue sample the electric field versus location along this antenna varies quite a bit, and the magnetic field also.

[Slide]

We can see in that supposed model of a cellular phone where the fields outside the body -- and the different colors represent different strengths -- induce different internal fields and the internal fields. Remember, E^2 is proportional to SAR. So, a cellular phone or another kind of near filed source that is held close to the body -- we have a hot spot, the highest SAR would be here close to the source and it drops off as you get further away. The whole body average would be taking the integral of all of the SARs at each cubic centimeter and averaging them out. So, if the local SAR might be 1 W/kg, the whole body average SAR could be 0.001 or less because you are averaging over the head, the feet and there is no SAR in most of the body. So, this is an important concept. SAR will always vary even if you have a uniform exposure.

[Slide]

We are getting close to the end and we can just say one or two more things. Measurement uncertainties -- the best that possibly can be done in a precision laboratory might be 0.5 dB or plus/minus 10 percent at each point in terms of SAR, local SAR. It is more typically, in the past, probably 20 percent or 25 percent uncertainty from one lab to the other.

[Slide]

This slide shows how you make measurements in a lab using an electric field probe. This is a 1.5 mm tip size where you place this in the in vitro sample or in a model of the head or in an animal's carcass and measure electric field inside the body. You can measure 5 microW/g to 100 mmW/gram.

[Slide]

You can also measure SAR with a small temperature probe and measure the delta T, delta temperature, over the number of seconds, and you can measure 200 mmW/g which is less sensitive than with an E-field probe. So, typically what will be measured in in vitro studies with small or low power sources is the electric field with an E-field probe but temperature probes may be used in a lab where you have an in vitro sample and a higher power source.

[Slide]

Finally, conclusions -- the SAR is the result of exposure to electromagnetic fields. Exposure refers to the external fields, electric or magnetic from a cellular phone. The SAR distribution is always non-uniform, very non-uniform even if you have a uniform exposure of the whole body. And, the uncertainty is at least 10 percent, plus/minus 10 percent and that is in the very best situation. Probably it is double that in any of the bioeffects research that has been reported to date for local SAR. Thank you.

DR. OWEN: Thanks, Howard. Next on our agenda we have a break scheduled. We had some extra time in there which is good because we started a little late. It is about 9:35 now, which puts us ahead of the schedule. I think it would be good to try and start at about 9:55 instead of waiting until ten o'clock.

[Brief recess]

DR. OWEN: To discuss <u>in vitro</u> RF exposure systems, I am happy to welcome Dr. C.K. Chou. He comes to us from Motorola Florida's Research Lab. He has a very long experience in engineering physiology and biophysics radiation research and, before going to Motorola, for several years he was Director of the Department of Radiation Research at the City of Hope National Medical Center.

<u>In Vitro</u> RF Exposure

DR. CHOU: Thank you, Russell.

[Slide]

My task this morning is to give you an overview of the RF in vitro exposure methods. I will concentrate on the dosimetry and some temperature control.

[Slide]

I will show you some examples, a lot of pictures. I will go through different systems very quickly, and emphasize SAR distribution. As Howard said this morning, SAR is non-uniform. This is also true in the in vitro exposure tissue cultures. There are different kinds of artifacts and I will point them out along the way. And, the temperature control, as I will emphasize, is important. And also the SCC28, the current safety standard setting subcommittee has engineering evaluation criteria and I will point that out in some of the parameters and some conclusions.

[Slide]

So, the in vitro exposure systems have different wave guides, shift line, coaxial line, radio transmission line, and wire patch cell, and some different horn antennas either applied in the near field or far field. I will show you examples of individual different systems.

[Slide]

When I first became a graduate student in University of Washington, in 1971, that was the time when

this whole issue came up because of Chuck Anderson's report in Washington Post on the Soviet exposure of our American embassy. So that was the time of my first project. Bill Guy said, well, the Soviet literature -- they put nerves in the wave guide and exposed them. They found no temperature rise but all kinds of effects on the nerve action potentials. So, we checked this system out to see if we could find effects like that.

But at that time we made some modifications because you don't see the nerve exposed in the air. So, we put this in a ringer solution to keep the nerve alive, and designed this wave guide with a material to match from air to ringer solution. Then we put the nerves either parallel to the electrical field or perpendicular to the electrical field and stimulated outside the chamber and recorded on the other side. In the meantime, we can also use some ports here to circulate through a constant temperature circulator to keep this tissue at a fairly constant temperature in that area to minimize the thermal effects.

[Slide]

So, this was the basic system. We exposed all kinds of nerves, and here is an example on a sciatic nerve from a frog. This was a very high power. The SAR was 1.5 kW/kg. That is a very, very high power level. When we put the circulator on, this is the microwave off and this is the

microwave on, and you don't see too much difference. You see a little bit of difference in temperature rise because of the limited pumping capability of the circulator. That is a one degree temperature rise. You see a little bit of a shift in latency but basically there is no difference until you turn off the circulator and the nerve deteriorates very fast because the temperature goes so fast. Fortunately, I turned the circulator right on and this thing recovers. So, we can see here it is pretty much relating to the temperature, not too much to the field even up to 1.5 kW/kg.

[Slide]

Also, you can extend this to different muscles. We tried a frog muscle, but this is a rat diaphragm muscle because it is very thin so you can keep the temperature very constant. We stimulate the nerve and you see the tension of the muscle twitch.

[Slide]

This is using a peak power, 220 kW/kg peak power, 1 microsecond, 1000 pulses radiation. This way you see a temperature only 0.2 degrees centigrade, and you can see there is a little bit of a shift there. So, biological systems, depending on what you measure, can be very sensitive to temperature variations.

Here I want to show you another example for CW 1.5 kW/kg. You see that there is a one degree temperature rise

here. This effect is also reproducible by turning the microwave off and changing the solution temperature by one degree. You see the similar effects due to the microwave. So, this gives very good evidence that this is related to the thermal aspect of the effect.

[Slide]

So, this system was later on modified by James Lane and he used a pipette to put cells in there and expose this way but it was basically the same design.

[Slide]

Mike Galvin, from NIEHS, in the 1980s, moved the wave pattern around and used a matching transformer here and exposed cells over here. He used this one as a control because by the time energy goes to here it is very small. So, one is exposed and one is the control.

This system was later adopted by Liu and Cleary, and they put a magnetic stirrer here to use external stirring to keep the cells floating inside so cells would not sink to the bottom. There is a problem with this system. The size of this is quite big and when you have a high power exposure it is very difficult to keep the inside temperature constant.

[Slide]

Jim Lane's group also modified the chamber. This is another variation. They put another inside chamber in to

put cells in here and do some recording this way. So, this is a different modification.

[Slide]

This is Ken Joyner, and when he was at the FDA, in the 1980s, he did work on alternated wave exposure system; put cell cultures in here and a temperature sensor to monitor there. If you want the details, you can ask Ken Joyner.

[Slide]

This is a tunable wave guide by Liburdy and Mangan. They have this tuning system to get energy into the cell culture that way. It is kind of complicated.

[Slide]

This is another method called stripline. Stripline uses a central conductor here and ground conductor outside. You put the cells inside there. This if from Wachtel, and you expose aplysia cells. This cell can have pacer cells in there that can be microelectral to record the neurological firing rate.

[Slide]

Here I want to point out is microelectral. When you put this very small pipette into a cell, and with highly conductive fluid inside you can cause this effect called intensification of the E-field of the tip of a very highly

conductive structure. This was analyzed by Bill Guy and put in the NCRP 1981 report.

So, he analyzed this structure. This is the ratio between the electrical field tip versus the electrical field on the side versus the ratio of this long shape, c and d, and if c and d is one, that means it is a circle. When this becomes a long needle, it goes to this side. So, you see that there are different curves for different solutions, different materials. When you use ringer solution it is almost flat. It doesn't affect the E-field of the tip. So, that is why we want to use that. So, saline or a ringer solution electrode. When you use a higher conductivity electrode this thing goes up, and you can see there are many orders of magnitude enhancement at the tip of the electrode. This can push whatever -- potassium chloride into the cell. This can have concentration effects and can affect the cell membrane and all kinds of transports, and all that.

[Slide]

There is one other possible artifact that you can generate by using this kind of high conductivity solution in the electrode. This is another stripline. We have a parallel plate here. This was University of Washington, in '75, using the cat spinal cord and trying to keep the circulator here to perfuse and cool the spinal cord and, in the meantime expose it to the microwave and also stimulate

the nerves and the CNE response change. The trouble with this is, because it is such a large tissue, it is very difficult to keep the constant in there.

[Slide]

This is the other system, called transverse electromagnetic cell, abbreviated TEM cell. You can commercially buy this as input and output. Inside there is a center plate called center septum, and you can open the door and put cell culture inside.

[Slide]

Then EPA and Blackman and Weil. When you expose chick brains inside here -- this is going vertically, and put chicken brains here and do all sorts of biological studies. At that time it was the calcium reflux study.

[Slide]

This is the one we are talking about, the project I worked on for WTR when I was at the City of Hope. We modified this TEM cell to make a temperature control because, as I will emphasize later, temperature control is very important. So, here are just the guts inside to show you from the inside. Here is a fan we put on top. Here are the different tubes. The tubes are flowing through the water, circulating through the wall of this TEM cell to keep this inside temperature constant. We have open windows here on all four sides, then we put this inside styrofoam, and

this will be all enclosed later and it will have a fan to blow air through all this with water circulation through this whole system.

[Slide]

So, after it is enclosed you can expose tissue culture in the Petri dish. There are four Petri dishes, two above and two below, and there is a center septum here. So, these two will be upstairs and these two will be downstairs, exposed to the signal you are putting into the system.

[Slide]

Bill Guy did the FDTD calculation. On this Petri dish we start a kind of orientation, and this is the bottom of the Petri dish. The monolayer cells will be on the bottom. This is distribution. You can see that it is quite uniform but still not perfectly uniform. You can see there are some higher SARs here but lower here. But other areas above this are very non-uniform and especially where you have meniscus they are very hearty. But if we have cells only down on the bottom, it is a good system to use.

[Slide]

That is the whole system we have here. Because this is a very low efficient orientation, we need a high powered generator to give enough SAR in the cells. This is all the associated monitoring system and the big circulator,

the circulator temperature inside the CO^2 to keep the cells happy in the cell culture.

[Slide]

But, unfortunately, when we started to work with the biologist and everything had to be according to good laboratory practices, so we said, how do we expose cells? Well, in their lab they have to use these test tubes. They cannot use the Petri dish. So, we had to quickly modify how we accommodate that. So, these are all the tubes they used and we had to use this method and we had to make this vertical and turn this thing 90 degrees, and have this mounted on the doors, three on this side and three on the other side to expose the cells.

[Slide]

Bill Guy did the calculations on the three tubes on the left side, and looking at it this way you can see the hot spot on the bottom and the cold spot here. If you turn it 90 degrees you can see that the center is cold and it is very hot on the side. This is because when the magnetic field goes into here you get an eddy current like this and that is why you have a hot spot on the side and a cold spot in the center.

So, if you have cells in this situation you have SAR variations in a very big range. So as a compromise, the biologist said why don't we spin the cells down to the

bottom one centimeter and, hopefully, we can expose the cells in this range and, hopefully, cut down the SAR.

[Slide]

Let's look at the computation. This is the whole tube; this is the distribution. It is very non-uniform. The mean is 4.7; the standard deviation is almost 4. So, this is not an acceptable way of exposing.

[Slide]

So, if you only limit to the bottom 1 cc volume, and this is the distribution, normal distribution of 4.7 and the standard deviation is 1.8. So, it is much better than if you have the cells all over the tube.

[Slide]

This is kind of difficult to see but it is mainly to show you the raw temperature data. We put 12-channel Luxtron fiberoptic sensors in the tubes and after a brief one-minute exposure we took the door out and shook them very vigorously, and then we measured the temperature afterwards and we can get a delta T here to compute the average SAR in the test tubes.

[Slide]

Then also the temperature, how do we set the temperature? We want the cells to be 37, plus/minus 0.1 or 0.2 degrees and we have to find out by trial and error what the circulator temperature is in order to get the cells at

37 degrees. For this particular power setting we need 35.8 degrees at the circulator to keep the cells inside at 37 degrees.

[Slide]

So, this was the system used for the study. We did dosimetry for Salmonella cells, mouse lymphoma and human blood cells because all these different mediums have a different dielectric constant. So, we have to determine the different dielectric constants first with a slotted line, with a very strict temperature control of 37 degrees and then we can calculate the SAR. This is the final number we used for the study. If you want SARs at 10 W/kg at the bottom of 1 ml of media, for this particular material of Salmonella you need 99 Watts in order to produce 10 W/kg in that cell. For mouse it is 15 and for human blood it is 17 W/kg. So, the temperature setting, for 10 W you need cooler temperature, 35.3 to keep the cells at 37 degrees. Even at no power input you see the 36 degrees, a temperature setting 1 degree lower than what you want in there because when you have this fan turned on to keep the air circulation there, the fan itself generates some heat. That is why you need to have this one degree lower. So, that is how strict the temperature control is that we have to do.

[Slide]

This is the final assembly of these two systems, and it was used at the North Carolina lab.

[Slide]

The next system is called the coaxial line exposure system. This was described by Bill Guy in 1977. This is where the cells go into 5 cc of volume. This is the inner conductor, outer conductor, and the electrical field between the two rings.

[Slide]

This is a teflon bottom. You can cover this here, put it in this region and cover the whole thing and you can expose cells like this. Energy comes from here and goes to the cell culture. These tubes are to circulate the silicon cooling oil to keep the cells inside there at a constant temperature. We were able to measure the temperature in there at 37 plus/minus 0.2 degrees in the middle of the chamber. So, that was described in 1977.

[Slide]

So, here I point out a very important biological experiment. Dr. Vernon Riley of the Pacific Northwest Research Foundation, in Seattle, was collaborating on the study. He used tumor cells injected into the mouse to see the latent period, how many days to see the tumor come up. So, this is normal, and it takes about six days to see the tumors start come up. He diluted the cells by ten-fold, one

log minus one, it takes about three days for the tumors to come up. So, this is the positive control. Then it put the cells at 43 degrees, and then because the higher temperature produced some cell damage, it took more than 14 days for the tumor to come up. This is for zero exposure, for sham exposure, no RF but put inside the same cell exposure system but no exposure, and that took almost 15 days to come up with tumor.

Then he started turning on the power at 4 V/cm and you see that there is a drop in response, and if exposed to 10 V/cm you see even higher, much shorter latency, and this looks like protective effects to the tumor cells, why there are more active tumor cells in this exposed group. That doesn't seem to make sense. At that time we could clearly say, well, this is due to non-thermal effects because you see a clear difference in the responses and our temperature was 37 plus/minus 0.2 degrees.

[Slide]

Actually, that is not true because we know the cells after a while settle down to the bottom. This is kind of a fuzzy picture; this is the only one I could find. So, we put a pump here to pump these cells, to force them to float in air instead of sinking to the bottom because if you have the cells at the bottom and we have this cooling oil in there to try to cool the temperature to 37 degrees -- for

different power levels you have to use a different temperature to cool it. So, when you have a high power you need a cooler temperature to cool it so the cells can have a thermal gradient that is different from the control sham exposure.

[Slide]

After we did that with the pumping we see absolutely no effect at all, even after 14 V/cm or higher we see no effect at all because we kept a good control on this temperature and there is no effect due to the RF for this particular experiment.

[Slide]

This is a system from Washington University, the radial transmission line. This is the upper conductor. This is the fan to circulate air to the inside. Opening this up, you can see inside, the T-75 culture flask in the radial direction. The energy comes from here and goes radially to the sound around the periphery of the absorber to absorb the energy.

[Slide]

This is the system they used. Pickard had a paper published earlier this year, and they analyzed SAR at the bottom of the cell culture. This is different elevation relative to the bottom and with different materials there,

and you can see there are different variations depending on how you do it.

[Slide]

This is a new one, published a few months ago from a French group. They call this wire patch cell. Essentially, this is two conductors. In the center there is a wire to produce a field. They put eight Petri dishes inside. They used a double container. The smaller one is inside. They said that this way they can improve the uniformity of SAR variation down to 30 percent.

[Slide]

Going to the next category is the antenna horns and the near field and far field. So, here is an example to show the antenna horn. They put the samples very close to the horn. So, this is called the near-field exposure, and they have a magnetic stirrer inside this. I am sure this is metallic and, of course, this is what generates all kinds of non-uniform SARs.

[Slide]

This is the far field. Harrison, Balcer-Kubiczek from the University of Maryland used the far field for the horn above and exposed this way, put a matching plate here and a flask inside temperature controlled bath -- inside the tissue culture flask, and they claim with 4.4 V/kg the temperature rise by 1.2 degrees centigrade. Even though

they have this circulator, it is difficult to keep the temperature elevation when you use the higher SARs.

[Slide]

This is another system. They have this air circulation. They have the test tubes like this, here, to expose cells parallel to the E-field. This is a report by Brown and Marshall.

[Slide]

This is another one with far field exposure from the top. They have tissue samples put here, and they have a rotating platform to try to homogenize the SAR, and also have air conditioned temperature blowing into this.

[Slide]

Marty Meltz had this thing inside a water bath, with a turntable with a tissue flask, and there are these styrofoam floats here. So, this exposure from the top is also far-field exposure system.

[Slide]

Bill Guy did the plane-wave exposure for tissue cultures like this flask. When the E-field is powered in this direction you see the non-uniform distribution with the hot spot on the side. The center is actually zero here. This is a very difficult thing to predict unless you do the analysis like using an FDTD calculation. If you are

measuring the SAR in the center you are missing the boat because the hot spot is on the side.

[Slide]

This is for our test tube exposure in the far field and energy coming this way. You can see that it is parallel to the E-field and you have the hot spot in the center. This is due to the far-field exposure.

[Slide]

So, we also did this comparison using different frequencies with plane-wave, the Temperature cell and test tube, T-25 flask. The numbers we tried to compare are the standard deviation, how many percent variation over the average numbers. You can see this test tube is pretty bad, about 100 percent, and also the others.

Here we used the Petri dish, as I showed you before, and it is very uniform. We have about 17 percent variation. If you use only within 3 cm inside the variation drops even further. But the one we were using is the long test tubes, and we chose to use the bottom 1 cc volume and the variation is about 41 percent SAR in there.

[Slide]

This is SCC28. That stands for Standards Coordinating Committee 28, subcommittee 4. We have engineer variation criteria. We are evaluating 100 papers in the

field to try to come up with a newly revised standard, and we will have a second meeting early next month in DC.

Here is the first group on the exposure parameters. We have to know the field characteristics. That is, polarization, the source of radiation, what kind of source you use, radiation characteristics, exposure duration, and we must know what the SAR is in the tissue, and Howard emphasized this before in his talk. We have to know the SAR, the induced current or E-field inside the tissue. What is outside doesn't really produce an effect inside. You have to know what is inside the body. The temperature reporting is also very important and we have to know the temperature in the samples and what method you use. Many people don't know that if you use metallic thermocouples or thermistors you can create all kinds of artifacts that can generate hot spots, and all that, and many people don't know about it. So, you have to use these RF non-perturbing probes for your measurements.

[Slide]

So, in conclusion, SAR of cells exposed <u>in vitro</u> varies with dielectric properties and depths of medium, and the size, shape and orientation of the flask, and the type of exposure system you use for your experiment.

Now, artifacts are very hard to recognize and eliminate, and temperature control gradient is very

difficult to detect, and temperature control is essential. In reporting RF effects you must consider all possible confounding factors. Thank you.

DR. OWEN: Thank you, Dr. Chou, for that very thorough explanation of the kind of problems that can arise in RF in vitro exposure systems, and the importance of considering temperature control.

I have some changes to the agenda to announce. Dr. Meltz is not present and will not be able to give his presentation. Dr. MacGregor has arrived and will be able to give the next presentation, which is entitled micronucleus assay -- regulatory aspects. Then, he will be followed by Dr. Verschaeve on RF genotoxicity and micronucleus studies.

While we are getting ready for this change in the agenda, I will mention very briefly something of what I know of what Dr. Meltz was going to talk about. Dr. Meltz is Professor in the Department of Radiation Oncology and Director of the Center for Environmental Radiation Toxicology at the University of Texas Health Science Center at San Antonio. He was unable to come -- he called me yesterday -- because of health problems in the family.

His talk was going to cover a wide variety of in vitro studies that have been done, topics that he chose for their importance to cell survival, cell growth and maintenance of genetic integrity. He also was selecting

endpoints that might be potential biological indicators of any adverse physiological or health effects, and one of the points that he had in mind to emphasize was that these various endpoints might usually be expected to be interrelated, such that if a significant change occurred in one biological endpoint there are likely to be changes in one or more of the other endpoints.

Again, I am unable to present his talk, but he was going to basically mention DNA strand breaks, DNA precursor uptake and incorporation, cell cycle progression, sister chromatid exchange, chromosome aberration induction, micronucleus formation, phenotypic mutation and gene activation. Obviously, several of these will be covered, I am sure, very well by the coming talks. The format of the talk that Dr. Meltz was preparing to give was basically to present some of the results in the literature in each of these, usually in a format where he showed an initial result and then followed that with a couple of other studies that demonstrate something that is common in the database that we have to work with, which is that there are a lot of conflicting reports in the database.

His bottom line conclusion was that it is important to note that adequate attention be given to the technical quality and biological relevance of even the peer-reviewed an published research. I think that that bottom

line goes well with the presentation that Dr. Chou just gave us on the difficulty of even in published studies knowing what the dosimetric aspects have been for in vitro studies, and similar problems can arise in the biological aspects of the experiments.

It looks like we are ready for our next speaker. I am pleased to welcome Dr. James MacGregor. He is from the FDA Center for Drug Evaluation and Research and, as I said, the title we have for his talk is micronucleus assay, principles and regulatory implications. Thank you.

Micronucleus Assay - Regulatory Aspects

DR. MACGREGOR: Thank you.

[Slide]

What I was asked to do was to provide some background on the micronucleus assay from the point of view of general principles of the assay, and the background and the regulatory use of the assay. As Dr. Owen said, I am from the Center for Drugs and so I am going to stay away from drawing any conclusions about the regulatory implications for devices which would be regulated by CDRH but, rather, I was asked to focus on the assay, how it fits in a general way into regulatory practice at a rather elementary level for those attendees who really may not be that familiar with the technical aspects of the assay and how it is used in a regulatory setting.

[Slide]

So, I thought I would start at the very beginning -- why our regulatory agency is concerned about genetic damage, and I think most people are familiar with the reasons for this. DNA ultimately controls all our cellular functions. It is the blueprint of life and if you affect systems that control DNA and its integrity you are likely to have cellular functional effects.

In particular, alterations in DNA have been associated with human disease, including cancer, and the nature of the lesions that have been associated with both heritable human biochemical diseases and cancer fall into several general classes of damage, normally referred to as point mutations or base substitutions or very small deletions within a gene, structural chromosomal aberrations and aneuploidies.

I am not going to go into all of the background on the health effects, but I think it is fairly well established that these classes of damage are associated with genetic alterations that have been associated with human disease outcomes. And, we know that many chemical and physical agents can cause these types of damage and so, as a regulatory agency, we are concerned about controlling exposures that may introduce these types of changes.

[Slide]

Much of the focus has been on cancer and I think the reasons for this are summarized in this slide. It is know mechanistically that mutations in oncogenes, tumor suppressor genes, etc. have been associated with the development of cancer. We know that the DNA repair process in the human is closely related to the development of cancer and that repair defects are associated with increased cancer risk. We know that as tumors develop genomic instability occurs within the tumors, and that genetic alteration is characteristic of the tumors. Again, we know that many kinds of physical and chemical agents have been associated with causing these types of effects, and in various kinds of animal model systems causing carcinogenesis and that has been tightly linked to the ability to modify DNA and cause heritable changes in the DNA. So, a lot of the focus of concern with genetic effects has been on the relationship between understanding the mechanism of genetic changes leading to carcinogenesis.

[Slide]

So, as I have already said, the concern has been with detecting these general classes of chemicals, and regulatory testing guidelines within a variety of types of regulatory agencies have been developed to identify classes of genetic alterations that have been associated with human and animal diseases and, essentially, these classes, as I

have said, are base substitutions, chromosomal deletions or additions, chromosomal aberrations and chromosome losses.

[Slide]

The micronucleus assay is a part of many of these regulatory schemes, and the reason is because it is a convenient screening test to detect agents that cause chromosomal abnormalities that lead to breakage, aberrations or loss of chromosomes.

[Slide]

The mechanism by which this occurs is illustrated in this slide. The principle of the assay is that when you have disturbance of the normal chromosomal replication and segregation process that leads to the lagging of either a whole chromosome or a fragment of a chromosome, these broken fragments or these lagging chromosomes may not be incorporated into the new daughter nuclei and may be left behind to form a small body that looks very much like a nucleus. It is a membrane-bound body that forms a small nuclear-like body, hence the name micronucleus. Because these micronuclei are very easy to detect and score, this assay has come into fairly widespread use in the regulatory community.

To think about the mechanism implies a number of things. If one has damage in a cell within the nucleus, the assay is detecting types of events that will do one of

several different things. One is that if there is a lesion in this cell that causes a break or if there are breaks within this cell, that is not visible within the cell until the cell goes through this division process. But if there are such lesions that lead to double-strand breaks within the chromosome, then one can get either chromosomal fragments without centromeres so they are not attached to the spindle of the cell, or it can give rearrangements that cause physical abnormalities that may cause physical impairment to normal segregation during the mitotic process, and these may lead to lagging fragments or lagging chromosomes, or disorders in the segregation process itself or within the spindle that may disturb the attachment of the chromosomes to the spindle and the normal function of the spindle may lead to lagging whole chromosomes that may give micronuclei.

[Slide]

Now, one of the things we will be hearing about in the meeting is data that are based on the so-called cytokinesis block method of micronucleus assay. This slide is simply a replication of the other, but depicting the process of division of a damaged cell using the cytokinesis block method where an agent such as cytochalasin would be added to prevent the cell from continuing through the cell

cycle but, rather, would be trapped in the first nuclear division without cytokinesis or division of the cell.

So, under these conditions damaged cells would go through their first replication and be trapped at the first division. Now, why would you want to do that? The reason you would want to do that is because the nature of most of these events is that they are cell lethal events. They are markers of classes of damage that may induce heritable genetic alterations, but the actual events being measured, which is the loss of a whole chromosome or a major part of a chromosome, generally is a cell lethal event. So, if you have normally dividing cell populations what happens is that when you damage a cell in the first-division-generated micronuclei and if that damage is not continuing, the damaged cells will be rapidly eliminated from the population. So, to maximize the sensitivity of the assay it is desirable to trap the cells within that first division where the damage is maximal.

[Slide]

Now, micronucleus assays have been developed in many different cell and tissue systems. The <u>in vivo</u> bone marrow erythropoietic cell assay is perhaps the most widely used. This is a part of recommended testing schemes in a variety of different regulatory centers and agencies. Many <u>in vitro</u> systems have been developed, and we will hear about

those systems during this meeting. Human lymphocytes or cultured mammalian cells in vitro or in vivo may be sampled and then cultured and micronuclei identified. Micronucleus assays also have been developed in other tissues, such as liver, intestine, skin, spleen, spermatocytes in vivo in the whole animal systems.

[Slide]

Basically, that is the rationale for using the test as a method of identifying those certain classes of genetic abnormalities. It is a convenient screening test, and I think there are some general considerations that we need to be aware of as we discuss the findings in this workshop and the implications of the findings.

First, scientifically we have to recognize that when we induce damage, the damaged cell must replicate and progress at least to nuclear division to degenerate a micronucleus; that the events that lead to chromosomal aberrations and, hence, micronuclei are generally cell lethal events; and that cells that contain them are generally rapidly eliminated from dividing cell populations.

So, we need to pay attention to the kinetics of the exposure in the sampling situation. In general, the frequencies are highest in the first daughter cells after treatment and then decline fairly dramatically in subsequent

generations. So, cells must be observed at appropriate times with respect to exposure.

Now, what does it mean when you have positive findings? Well, I think if one has positive findings and if those findings occur under conditions of exposure that are relevant to in vivo exposure situations, then those findings would raise significant concerns about carcinogenic and possibly other health risks depending upon the exposed tissues.

I think it is also important to keep in mind that mechanistic studies might be indicated to fully understand the significance of the finding. As I showed you, there are a number of different mechanisms that can generate a micronucleus, and the implications, the risk implications of an agent that is developing DNA strand breaks may be a little bit from those that disrupt spindle assembly, for example. They may have different types of dose-response curves, risk extrapolation and so on, and in a short introduction I don't have time to go into it, but there are ways to follow up on these studies and to identify the nature of the damage and the type of damage that has been induced.

So, basically, those are my comments. The idea was just to give a very brief background on the mechanisms and some of the kinetic considerations that I think are of

concern, and how the assay really fits into general regulatory schemes. Then, as far as specific application within the radiofrequency energy area, that is something that I am not personally involved in but that CDRH would be able to comment on if you wanted to discuss that further, later in the workshop.

DR. OWEN: Thank you very much, Dr. MacGregor, for a very helpful introduction to the specifics of the assay and to its use in regulatory assessments.

Our next presentation is by Dr. Luc Verschaeve on RF genotoxicity and micronucleus studies. Luc has long experience in genetic, carcinogenic and teratogenic effects of radiofrequency field exposure. And, I would ask him, first of all, to give me two things -- a tutorial on the pronunciation of your last name, and also to explain to everyone what VITO, your institution, stands for.

RF Genotoxicity and Micronucleus Studies

DR. VERSCHAEVE: Well, the pronunciation of my name is Verschaeve. But I think it is only in Flemish countries that it is pronounced like that. So, I am used to having a lot of different pronunciations. Sometimes, when some people call me, I don't even understand that it is me that they want.

[Laughter]

So, don't worry; I think that you did it very well. Then VITO, VITO is the institution where I work. In free translation, it is the Flemish Institute for Technological Research. It is an institution where we have, in fact, research on three different main topics, let's say. It is new materials; it is energy and it is environmental studies. I belong to the environmental department, and most specifically to the toxicology department where I am working in a genetic lab, and let's say that most of the things that we are doing are related to environmental pollution, and radiofrequency fields are one of the things that we are doing, but it is not only that.

So, let's come to the discussion of today. I was asked to give a short overview of the studies that have been performed so far in the field of genetic toxicology of radiofrequency fields. Now, as you probably know, there are at this moment I think about 100 or 150 different studies that have been published so far. So it is, of course, not possible in 20 minutes to give an overview of all those individual studies.

[Slide]

So, I would like to start by giving some review papers for those who are not acquainted with the topic. If you want to have more information, you can consult those papers. They are relatively easy to obtain.

The first paper is from Brusick, which was published about two years ago in Environmental and Molecular Mutagenesis, and the second one is from myself, about the same period, published in Mutation Research. So, there are only four if you want to have more detailed information.

[Slide]

This morning there were also two expert panel reports that were mentioned. The main conclusions were already mentioned too so I will not do that. But I can maybe say that both reports can be downloaded on the Internet. So, if you want to have them, they are completely available. They both have about 150 pages so they are quite important reports not only, of course, on genetic toxicology but on all health aspects of radiofrequency fields and, more specifically, on mobile phone frequencies.

So, the first one is the Royal Society of Canada, which was mentioned, and the other one U.K. Independent Experts Group on Mobile Phones. That was made available only two months ago. So, if you want to download it, it is really easy to do.

[Slide]

Now I will come to genetic toxicology of radiofrequency fields. As I said, I can only give some generalities, but I think it is important to say that from the 100 papers that are published so far actually most of

the studies are involved with frequencies that are not mobile phone frequencies. There are a few studies on mobile phone frequencies but most are other frequencies, and also the exposure conditions are usually different from what you can expect when you are using a mobile phone. So, I think it is important to say that, even if I think that you can more or less consider that the results aren't relevant yet.

More important than this, as was already said this morning, is that we all know that radiofrequency fields can have thermal effects. When the exposure level is high enough you will induce heat in cells or you will induce heat in organisms, and it is well known, for years, that just heating can cause genetic effects. You can have micronuclei by heating; you can have teratogenic effects, bioeffects, and so on just by heating. So, it is important to know that a lot of studies, and probably most of the studies that have been published so far, were studies where the exposure level was relatively high and where you can say that a thermal effect is probable, if not for sure. Of course, there are also other studies and other effects possible that are not thermal effects but in many cases we have to deal with publications that are on thermal effects.

[Slide]

I think it is important to say that because when we look at the data that is published -- this is a table

that I made from the paper of Dave Brusick. It is only part of the data but I think it is enough to show what I want to say. That is, when you look at the different studies that are already published now -- and this is for '98 so there are a few more now -- you see that, for example, there are 24 investigations published on microbial systems, and only one gave a positive response, and by positive I mean that some genetic effect was identified. So, in this case about 4 percent of the investigations with microbial systems were positive.

But when you look at in vitro cytogenetic studies, from the 32 studies 12 were positive which is a little bit less than 40 percent. You can see the figures for other tests but I don't give them all. But, for example, dominant lethals and in vivo cytogenetic tests score relatively high. About 50 percent of the studies show some genetic effect.

When you look at this table, you can think, I believe, that, indeed, radiofrequency fields can pose some problems. A lot of studies are positive. Most are negative but, yet, a lot are positive. But you have to look at the individual studies. When you do that, you will see that, for example, especially for dominant lethals and in vivo cytogenetic studies, most of the studies are clearly studies where the exposure was thermal. So, I think that the conclusion can be that most of those positive studies are

due to heating effect and not to the radiofrequency fields as those that you can have when you use a mobile phone, for example. So, this table is, in my opinion, rather misleading. I think that heating is the main reason for the positive effects even if most of the studies remain negative.

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Now, with what I said about the radiofrequency fields in general I tried to give you some idea about studies that are performed on mobile phone frequencies. I give you a table of most of the studies that are published so far. So, you can see that there are studies on different endpoints. I only give a table for cytogenetic studies here. There are studies on chromosomal aberration, sister chromatid exchange, micronuclei and some others. Different cell systems were used. Most of the studies here are <u>in vitro</u> studies; different SAR values. And, you see that there are different outcomes but most of the outcomes, again, are negative but there are some positives. There is increased chromosomal aberration here. I had better not talk about the last study because it is a study that is not completed and it should be evaluated further. But, anyway, there is one positive study here with an SAR of 1.5 W/kg which, I believe, is still thermal.

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I go on with the table. You see some other studies here. Again, most of them are negative but there is one positive here. This study by Phillips, in 1998, was performed under conditions that can be considered non-thermal, and it is a positive one where you have a decrease in genetic effect when the exposure level is low but an increase with a high exposure.

So, again, when you look at studies on genotoxicity of mobile phone frequencies, you can more or less have the same conclusion as for radiofrequencies in general. Most studies are negative but there are some positive ones, although you can mainly think that it is due to heating but not always.

Another thing is that sometimes people think that, okay, radiofrequency fields or mobile phone frequencies are not genotoxic themselves but they can have some epigenetic effect. They can, for example, enhance the effect of something else. This was already investigated, mainly by the group of Meltz in San Antonio, who should be here today. You can see that all his studies give a negative outcome for different frequencies, for different cell types, different genetic endpoints and also different chemical or physical mutagens that were used in combination with radiofrequency fields -- for example, mitomycin C, adriamycin, U-violet proflavine. Combinations were made. The cells were exposed

simultaneously to the radiofrequency fields and the chemical or physical mutagen and always the results were negative.

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But, again, as for everything, this is not always so. There are a few studies where positive findings were obtained. The first one is a conference report, and I am not sure I can mention it because I don't have too many details about that study and at least, myself, I have no details about dosimetry so maybe we are clearly in heating conditions, I don't know. Anyway, they found an increase of the effect of ethylmethane sulfonate when cells were exposed in combination with two radiofrequency fields.

The second study was performed in my lab and we found the same thing. The effect of mitomycin C was clearly increased in a very reproducible way when cells, in this case human lymphocytes, were exposed to 954 MHz radiation and then, afterwards, to mitomycin C. So, the exposure was not simultaneous but first radiofrequency fields and then cultivation of the cells in the presence of mitomycin C.

We looked here at sister chromatid exchanges. We have done this study for eight or ten different donors. We always found the same reproducible effect. So, we are sure that this result is true. But there is a problem, and that is that if we repeated that study with lower SAR we found more or less the same result but much, much less clear. It

was more or less borderline. We could think here that this is because the SAR is lower. So, maybe that is the reason. When the exposure level is lower you have a lower response. But most surprisingly, we repeated it again for 900 MGz with mitomycin C, and we did a study with a power output from zero up to 50 W which is an SAR of 0 up to 10 W/kg, and we always got negative results. So, it is completely in contradiction with the study that we performed before.

I have no explanation for that at this moment. The only thing that I can say is that here we went from 0 dose to relatively high doses that are clearly thermal and we don't find anything anymore. The difference can be the exposure system. Here is the M cell that was introduced a couple of minutes ago. Here it was just exposure close to the antenna from the GSM base station.

So, again, I think if you look at all those studies on synergistic effect, most are negative but there are some, let's say, puzzling data that still remain and that maybe merit further attention.

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I will also say something about a study that is not published yet, but we performed some biomonitoring studies for, at this moment, more than 40 subjects that are occupationally exposed to radiofrequency fields, and mainly from GSM mobile phone type. We found no increase in

chromosomal aberration frequency; no increase in sister chromatic exchange; and no increase in DNA content. So, all this was completely negative. Maybe this is an important study because it is a real-life situation. Unfortunately, we have not investigated micronuclei so far so it would be interesting to see what this would give.

[Slide]

Now I come to the studies on micronuclei. Here are the main investigations on this subject for in vitro micronucleus test. You can see that there are again, as always, some positive and some negative results but it is quite surprising that most of the studies are positive in this case. You have, for example, four positive studies here and only one negative study. Again, of the positive studies some of them may be considered due to thermal insults. This one, for example, was a pilot study that we performed in our lab. The SAR is 75 W/kg I think, not 76 but it doesn't matter. Anyway, it is high. It is clearly thermal.

In this publication we also used a metallic thermistor probe, and as was said a couple of minutes ago, this was also leading to hot spots probably. We have repeated the study without the metallic thermistor. We still find increased micronucleus frequency but the SAR is still high.

Studies by Garaj-Vrhovac and her collaborators in Zagreb are also positive. They claim that the exposure situation was non-thermal but when you clearly look at the data, most probably dosimetry was not well performed and the exposure is thermal.

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So, in vivo studies -- you can see in rodents different frequencies, different SARs and different cell types that were investigated but, again, positive results and negative results.

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Some may be due to thermal effects but not all, and what may be important here is that some studies were performed on, let's say, normal life exposure situations. For example, this is not mobile phone frequency but involves living close to and in front of a radar station and you have clearly increased micronucleus frequency in the blood lymphocytes of those living there who have, let's say, normal daily life exposure.

We also have a positive study here, again by the group from Zagreb, where there is a clear positive response in micronuclear frequency in occupationally exposed subjects.

DR. CHOU: What is the difference between the last one? One shows positive and one shows negative.

DR. VERSCHAEVE: The difference is just that they did two different studies using two different groups of occupationally exposed subjects. So, I can consider that probably the exposure is different. The only problem is that in those two studies there was no real dosimetry. You can only say it with people who were exposed during a given period of time due to their occupation, but you don't know how much the exposure was. So, it is most probable that the exposure here is less than the exposure here. But it is not really documented because they have not done dosimetry.

So, I think actually I can stop here just by saying what I said in the beginning of my talk, that is, when you look at all the studies that have been performed so far, most are negative and the overall conclusion is that radiofrequency fields are not genotoxic.

When you look at those studies that give positive results, most of them are clearly due or probably due to heating effects, but when you look at micronucleus frequency compared to other types of genetic endpoints, then you see that there are a few more studies that are positive than negative in what I may call here real-life exposure situations. So, I think that is probably the reason why we are here today, to discuss what the relevance of this is. I will stop here. Thank you.

DR. OWEN: Thank you. With that, we are at the conclusion of our introductory presentations, and because of missing one talk and other things we are a little bit ahead of schedule but not too far ahead of schedule.

Discussion

The purpose of the discussion slot in the agenda right now is to obtain any clarification that is needed on the background information that has already been presented this morning so that we will be as fully prepared as possible for the data presentations this afternoon. So, i would like to ask at this point people in the working group whether they have questions they would like to pose to others of the working group that made the presentations this morning.

DR. TICE: I have one thing that Luc might want to mention or talk about a little bit. On some of those studies that he quoted or looked at that were in vitro where they looked at micronucleus, they also looked at chromosome aberrations at the same time. If I remember, at least in the studies done in your lab, they saw an increase in aberrations under the same conditions that they saw an increase in micronuclei.

DR. VERSCHAEVE: Yes, that is right.

DR. TICE: Then, one of the studies that was done by the group out of Belgrade, I guess --

DR. OWEN: Zagreb.

DR. TICE: Yes, Zagreb. They also looked at the micronuclei based on size distribution to try and see if they could understand the mechanism of formation, and they concluded that the majority of micronuclei were probably caused by acentric fragments, which also went along with them finding an increase in aberrations in their exposures. So, at least in those particular sets of experiments, there is a concomitant increase in both aberrations and micronuclei, and it looks like the mechanism that is operational is acentric fragments. And, that is probably important from the standpoint of trying to understand mechanisms.

DR. OWEN: Howard?

MR. BASSEN: Yes, Dr. Verschaeve, in your talk you stated that probably most of these positive effects on micronucleus formation are due to temperature rise or thermal effects, and the SARs on these are known with some degree of uncertainty.

DR. VERSCHAEVE: Yes.

MR. BASSEN: My question is in humans there is a temperature variation, especially due to metabolic activity, heat stress. What is the normal range of human temperature rise, and why doesn't that cause micronucleus formation compared to RF, or does it?

DR. VERSCHAEVE: If I understand your question, well, you say why doesn't normal increase in body temperature give micronuclei and heating effects by radiation? That is what you asked?

MR. BASSEN: Why does temperature rise due to normal metabolic activity not cause micronucleus formation in the human? These studies, I understand, are in vitro. So, I am just trying to distinguish between RF-induced micronucleus formation that may be due to temperature rise and to differentiate that from is there micronucleus formation in humans due to temperature rise from other sources?

DR. VERSCHAEVE: I am not sure. I don't think I can answer that. I mean, as far as I know, nobody has done micronucleus tests in people with, let's say for example, some high physical activity where body temperature increases. I don't think this was done. When physical activity is high you will find increase in DNA damage --

DR. FENECH: Excuse me, there have been studies following exercise with the micronucleus assay --

DR. VERSCHAEVE: Maybe.

DR. FENECH: -- and increases have been reported.

DR. VERSCHAEVE: And there was an increase --

DR. FENECH: Yes. There was at least one German study that I am aware of.

MR. BASSEN: But it is not necessary due to thermal --

DR. FENECH: No, it is not necessarily due to thermal. It could be oxidative or a combination of both.

DR. TICE: I might mention that at least in animal studies there is one in vivo study putting hyperthermia --

DR. VERSCHAEVE: There is one, yes.

DR. TICE: -- into mice and showing an increase in micronuclei --

DR. VERSCHAEVE: Yes, that is why I said it is documented that heating will increase micronuclei.

DR. TICE: Also, when they do hyperthermia for tumor treatments there have been associated increases in aberrations --

DR. VERSCHAEVE: Yes, but that is also radiation induced.

DR. TICE: Yes.

DR. VERSCHAEVE: So, you cannot say it is normal body activity.

DR. HOOK: But with normal body activity you are really talking about a three degree increase in body average temperature. You don't usually see these effects of micronucleus until you get up to about 40 degrees C in animals. So, that is outside, I think, normal fluctuations.

DR. VERSCHAEVE: Yes.

DR. HOOK: You would be in a high fever state.

DR. VERSCHAEVE: With high fever you have micronuclei.

DR. HOOK: Yes, with high fever there is some evidence.

DR. WILLIAMS: In those studies was the micronucleus pattern with any particular type of chromosomal aberration?

DR. HOOK: The ones that I have seen, as opposed to what has been reported here so far, are showing that it is likely to do with whole chromosomes rather than acentric fragments. That was based on in situ hybridization looking for what was in the micronuclei and they are finding whole chromosomes.

DR. TICE: It looks like another important point, at least from looking at our data and trying to explain it, is that all the hyperthermia effects are when cells are proliferating. Hyperthermia is there during cell proliferation and you are getting abnormal cytokinesis, abnormal disjunction, and that seems to be the primary cause.

DR. HOOK: But do you see it only when cells are near mitosis when they are heated or when they are heated in other parts of the cell cycle?

DR. TICE: I don't think the way the experiments are designed can really -- because, you know, you are exposing a body or exposing cells and then you are doing sampling and you don't even know if there is a delay, but Joe might have a comment.

DR. ROTI ROTI: Yes, I was going to say that we haven't specifically looked at micronucleus formation but most of the nuclear damage that is made permanent during heat involves cells having to traverse S-phase shortly after the heat shock or during the heat shock. Then, the damage is manifest in mitosis. So, it is not really damaged during mitosis but, rather, during S-phase.

DR. VERSCHAEVE: Blood lymphocytes were exposed -- I mean, you take blood, you expose the cells and after the exposure you grow them and you still have micronuclei.

DR. ROTI ROTI: Right.

DR. VERSCHAEVE: So, it is not only during S-phase.

DR. TICE: So, there can be a delayed response.

DR. ROTI ROTI: I will just comment on that. If you use higher heat exposures, then post-heat transit of S-phase can fix nuclear damage. If you have rather modest hyperthermic exposures, then cells have to actually be transversing S during the exposure.

DR. MOROS: I have a general question for the panel. Has anyone characterized in any cell line what the thermal dose is necessary to see micronucleus? What I mean, if you heat for ten minutes what would be the temperature? If you heat for thirty minutes what would be the temperature that would cause it? Because we really need that piece of information to judge any RF study, either prospectively or retrospectively. We need to know when the MN appears. If that has not been done, then this is definitely something that needs to be done.

DR. FENECH: As far as I am aware, it hasn't been done systematically.

MR. BASSEN: Could you repeat your last sentence? You said we need to know when the MN -- what?

DR. MOROS: It is sort of like charting the territory before you go into it. For several cell lines we need to know micronuclear formation as a function of thermal dose. Thermal dose, as you may know, is a combination of time and temperature. The effects of 40 degrees may not appear in five minutes but may appear in half an hour. You know, the effects of 90 degrees may appear in a couple of seconds. So, for temperatures that are relevant to the radiofrequency experimental field, we need to chart the territory and we need to know micronuclear formation as a

function of thermal dose, and it appears to me that that is not available.

DR. FENECH: And, of course, if you are using the human lymphocyte test system, it would also apply to inter-individual variation and the response to heat, of which we have no real idea at this stage.

MR. BASSEN: So, this would be a sham type of exposure with temperature elevations? Is that what you are suggesting to do?

DR. ROTI ROTI: I guess I can answer that because we are doing some sorts of studies along these lines. In a sense, since we always talk about thermal artifacts or thermal effects confusing the non-thermal RF effects, what we have been doing with some of the biological endpoints is characterizing low-dose thermal effects for that endpoint in terms of time at a given temperature that would be typical of something that might occur in the field without you detecting it.

So, what we are saying is that heat could be used as a positive control. That is one need to do a time temperature characterization. But another need is to characterize it as a potential artifact and what effects on micronuclear formation are there for half a degree for 30 minutes, or for a degree for 15 minutes, or something like this.

MR. BASSEN: In the absence or RF exposure?

DR. ROTI ROTI: Yes, in the absence of RF exposure.

DR. MOROS: I would like to say something else. I am a biomedical engineer and medical physicist, and I have worked in the field of hyperthermia for many years too so I have some practical experience with thermometry and those experiments in the biology lab. So, I have some feeling for what the temperature trajectory is maybe for a group of cells in whatever container you have. And, one thing that never comes up -- well, I shouldn't say never but almost never comes up in the publications that I review is what happens before the cells make it to the irradiators. Most of these cells are actually cold-shocked, and nobody has really characterized what is the impact of actually lowering the temperature of the cells from 37 in the incubator to maybe 20, maybe 15 depending on the ambient temperature. There are evaporated losses during this trajectory that may have an impact. We don't know; we haven't looked at it. And, that may explain, I may say, some of this inconsistency in the results -- sometimes positive; sometimes negative for the same basic experiment.

DR. ALLEN: Is there any information on whether by reducing the temperature this would impact heat shock response or other kinds of stress response?

DR. ROTI ROTI: Absolutely, there is a lot of literature on cold-adapting cells before you do hyperthermia on them because if someone is treating a skin tumor it is a very different thing because the skin is colder than the internal surface. So, there are lots of different heat-shock responses -- heat-shock response based on the delta temperature, not on the absolute temperature.

DR. HOOK: And there are reports in vivo that hypothermia also induces micronucleus --

DR. ALLEN: This could be through a lowering of the stress protein response. It is possible.

DR. HOOK: The time frame is a lot shorter though. I mean, normally you don't have the cells sitting out for a long time as opposed to the time that you are exposing to what might be a hyperthermic condition.

DR. FENECH: I would like to ask for some clarification on this question of the thermal effect being considered as a confounding factor. We have had one or two experimental designs shown to us where the surrounding water temperature is reduced to make sure that the temperature in the culture tube is about 37 degrees.

The point of clarification I would like here is in terms of the relevance to the in vivo situation because in the in vivo situation we have blood circulating, let's say, around the tissue at 37 degrees. So, one could argue that by

lowering the temperature of the circulating water you might create an artifactual system that doesn't quite reflect what is happening in vivo in terms of the circulating fluids around the tissue you are studying. I was wondering what the response to that sort of view would be from the panel.

DR. CHOU: Well, that is exactly what we want to do. Even in the body, to keep it constant at 37 -- if you control the temperature you will see something relating to RF alone. So, that is the whole purpose of trying to control the temperature.

DR. FENECH: If you have a tissue close to the source of radiation in the body, the blood flowing around it presumably would be at 37 degrees. Is that correct?

DR. MOROS: Actually, that is not always the case. Blood temperature, whether it is arterial or venous, changes depending on what part of the body you are talking about. The blood in your hands may be 25 degrees at this moment in this relatively cool room. Now, your internal temperature inside the body cavity is always around 37 degrees but not anywhere else. Your ears may have very low temperature.

DR. FENECH: Are temperature measurements being done --

DR. MOROS: Actually, not in the field of RF that I know of, but there is a very large body of literature in

terms of human regulatory responses because of the space program. So, it is out there.

DR. CHOU: Well, there is a study on that but it is not published yet. They measured the temperature of the cell phone at the ear, and the conclusion is that most of the heat is due to the battery heating. You can terminate the RF and the temperature rise due to the phone heat conduction --

DR. MOROS: Sure.

DR. CHOU: -- compared with the antenna there, there is not much difference. So, that means is that most of the heating is due to the battery heating.

DR. LOTZ: What I thought Michael was asking was the difference, and while blood temperature is going to vary considerably depending what part of the body you are talking about in a given part of the body, under stable conditions it is going to be relatively constant. Quite so, in fact. And, what I thought Michael was asking was the contrast in that between the situation we often apply in the in vitro laboratory setting where you offset the circulating bath temperature to compensate and remove the heat that the RF might create in an effort to maintain an isothermal condition, if you will, and that that is different. That is what I thought Michael was bringing up.

DR. FENECH: That is the brain offset.

DR. LOTZ: That is right. The body doesn't offset for a particular tissue. If anything, if there is significant heat deposited, the blood temperature will go up in that region as it takes away that heat.

MR. BASSEN: In terms of head and brain especially the body thermoregulates that most of all. So, any heating that is induced in the brain is immediately equilibrated to maintain 37 degrees in the brain. So, that would be similar I think to an in vitro exposure system where you are trying to maintain the fluid and culture media at 37 degrees. By cooling, or whatever means, it would emulate the blood.

DR. HOOK: Not to minimize the argument, but the designs of our exposure systems are not meant to try to mimic the human situation. What we are just trying to do is keep the cells at 37 degrees because we know that if we let it go up we can have effects. So, that is the reason.

The other thing is that I would think that in part it is the gradient that is the important issue here. In that case, then we might be talking about something that is a lot closer to human situation. There is a thermal gradient there in both cases and that might be the point that needs to be considered rather than which one is higher or lower.

DR. MOROS: I agree. There are two issues that are important from my point of view. As the SAR increases,

then the gradients are going to be higher. So, your cooling method has to be more effective. Then, when you start getting into high SARs, and I probably mean about 10 W/kg, you start talking about thermal temperature within your sample and, again, that is something that has not been charted, not been studied.

But it is important to keep in mind that if you do a point temperature measurement, it doesn't mean that your entire sample is at that temperature, especially as the SAR starts going up and your thermal gradient is going to be more and more drastic. So, that is just something to keep in mind.

DR. MACGREGOR: I have two questions and I apologize if these were covered before I arrived, but one was in those positive studies, how do the radiofrequency energies in the studies compare with those that might actually occur in the exposed human in vivo?

Then, the second question is do exposures that could occur in the human, are they high enough energy to change the temperature, or is it established that there are no temperature changes in exposed humans?

DR. VERSCHAEVE: Maybe I can say that at least for those studies where there is, let's say, real-life exposure situations, like the cows under radar stations and some occupational subjects, apparently there is no increase in

body temperature as far as I know, but I have no details of many of those studies. So, I can only say for some that I know.

DR. OWEN: I guess the other factor that plays into that is that the existing safety guidelines are designed to limit temperature increases. So, that would be part of the reason that you wouldn't see a lot of real-life exposures.

DR. ROTI ROTI: Can I come back to a question that was raised when we had some discussion because I think there is a research philosophy that needs to be put on the table as part of an answer to the question of the real -- the fact that there is possibly a thermal rise in the human exposure situation.

To begin with, I think there is debate regarding are there robust, reproducible, non-thermal effects of RF radiation. And, to answer that question first we really need to have the constant temperature exposure studies done. If and when a robust, reproducible effect is established, I think then it is very appropriate to go ahead and ask is there a synergy with a small temperature rise because that is important to the question you put on the table. But I think you really need to define the effect first, and we have done studies like this with heat-shock factor activation, as a matter of fact. We actually did this with

915 MHz radiation to see if just heating with microwaves alone was any different than heating with a water bath and HSF activation. So.

DR. MACGREGOR: And?

DR. ROTI ROTI: And there wasn't any difference.

DR. MOROS: Going back to the question over here, Dr. MacGregor, in the cellular phone frequency range, and I think I am talking about 900 MHz, there was a paper this year in the European Journal of Physics and Medicine and Biology, by a group from Der Utrecht who have developed in the last 15 years or so a very sophisticated thermal model for use in hyperthermia cancer treatment. They applied that model to the case of the cellular phone against the head for heating of the brain, and they showed minimal heating. I think the maximum was around two-tenths of a degree Celsius. That is the best thermal model, most complete thermal model -- it includes blood vessels and everything else -- that exists. So that speaks volumes to me, that increase in brain tissue temperature due to cell phone exposure is minimal.

DR. CHOU: Yes, that is also true for the Japanese paper and also an Italian paper which also say the same thing, using thermal modeling with FDTD calculations.

Then, in addition to that, I also mentioned the measurement -- when you put a phone near your ear, even your

office phone, you are going to block the circulation and press on your ear and that can increase the temperature by several degrees. So, comparing with RF absorption, it is less than 0.2 degrees. So, relative to the other effects it is a very small difference.

DR. OWEN: Dr. Lagroye, did you have a comment or question?

DR. LAGROYE: No.

DR. MACGREGOR: I guess I am still not entirely clear on my first question, that is how the in vitro exposures correlate to the human in vivo frequency. Are they much higher or the same order of magnitude?

DR. VERSCHAEVE: Yes, the in vitro studies, indeed, usually are much higher than in vivo. If that is what you want, that is, indeed, the fact.

MR. BASSEN: I thought you had SAR levels of 4 W/kg where you saw effects.

DR. VERSCHAEVE: Yes.

MR. BASSEN: And the cellular phone limit is 1.6 W/kg in the maximum hot spot. So, it is not that far away. In the head that is the maximum in any place, and most places it is much lower.

DR. OWEN: The results presented this afternoon also will bear a lot on that question. So, it might be

something that we can go back to in the discussion after those presentations.

DR. FENECH: To the radiophysicists, can I ask is there anything known about the differences in the dielectric properties let's say of the nucleus as opposed to cytoplasm of the cell, and do they expect differences?

MR. BASSEN: Usually the cell is so small compared to a wavelength that the dielectric properties are not considered. There are significant changes between the membrane and the nucleus but they are too difficult to measure. You can't measure those. Because they are so small you can't extract just those elements out of the cell to measure the dielectric property. So, we are talking about microscopic dielectric properties that are known.

DR. WILLIAMS: One question, there seems to be a consistency among the people here when they talk about temperature patterns, that they are well determined. I suppose my question is over what physical domains and time domains do we feel we really understand the induction and diffusion of heat within tissue? For instance, there is some data saying that pigmented tissue may absorb more strongly and may, for a very short time, then be at a more elevated temperature compared to other tissue, say, in the eye. I have not worked in the field enough to interpret whether discussions here are based on a general appreciation

that, yes, when we see these pictures of temperature profiles that they are quite exact. Are there calculations and then point measurements to confirm them?

DR. MOROS: I have not really put my finger on your question.

DR. WILLIAMS: When we see these pictures of temperature profiles, are they frozen in time? Are those averaged? Over what domains? I get the impression that we think that these are a general mechanism in which the heating is fairly uniform and there is something about the shape and structure in the field that produces a difference in temperature. What I am asking is over what time domains and over what physical domains are these measurements valid?

DR. CHOU: I think I can answer your question. The pictures I showed with the different colors and all that, that is the SAR distribution. That is the instantaneous time capture of energy deposited in different areas. Then after that there is a temperature rise depending on the thermal diffusion and all other things. In the body blood flow and everything will be different. But that is where energy goes. So, that is independent --

DR. WILLIAMS: Is that a calculation or --

DR. CHOU: That is a calculation; sometimes a measurement. It depends, yes. Most of the colored pictures are calculations.

MR. BASSEN: But the temperature profile versus time at any point is highly variable for the given SAR. The SAR is the rate of temperature rise.

DR. WILLIAMS: I guess my question then would be when you say one point for one temperature, what domain are we talking about? In other words, are we talking at the level of cells? If you have cells contiguous in time? Or, are we talking about microseconds?

MR. BASSEN: We are talking about seconds. Taking a snapshot every second, you would see the temperature elevate for a given SAR unless you cooled it with some external means. So, SAR is the rate of temperature rise. So, given the fact that you can't measure it closer than a few millimeters in spatial resolution, you are watching a millimeter space elevate a few tenths of a degree over seconds.

DR. OWEN: Essentially in non-living tissue.

MR. BASSEN: And you have to cool that down. So, there is some equilibration.

DR. CHOU: But this is also important in terms of temperature control. We are not just talking about final temperature being the same; it is the RF exposure. As I also pointed out before, time variation during the history is also important. RF heating is very difficult to mimic by ordinary, conventional water heating and all that. So, this

is going to be another variable. Your history can be totally different and cause different effects in the biological systems.

MR. BASS: But over, say, five minutes everything is going to reach thermal equilibrium and you are going to get sort of a temperature profile that is going to be non-uniform in your sample cell.

DR. WILLIAMS: Well, your five minutes, is that significant or insignificant? What temperature for what period of time?

DR. ROTI ROTI: I am going to mention a couple of things. We don't know yet. I gave a talk a little while back on thermal effects, and people used the classic Dewey-Saparetto time-temperature conversion equation and created a line in that space of time temperature and said anything below this line is not going to have any heating, and we found out that HSF activation actually falls below that line. But, that kind of work hasn't been done.

I think that there are two things -- I haven't give my talk yet so I am kind of running ahead but I would like Eduardo to comment on the engineering because you were talking about calculations versus measurements, and we did the calculations or our engineering team did the calculations. They also did thermographic and temperature probe measurements. I don't remember quite how finely they

mapped, but they spent a long time walking across the T-75 flask bottom with a probe in the field. Yet, we have been working on, and we are just getting started on it but I would like to do more work on it and it would probably be part of any of these projects, and that is a biological thermometer that could be utilized in real time. And part of that is activation of HSF but we also have an HSP-70 construct tied to a GFP. Once that is stably transvected into 3T3 cells, those can be grown in a uniform monolayer so that you could try to activate it. We just don't know anything about the sensitivity yet. It may not be sensitive enough for this kind of fine mapping of temperatures. But that would be something that would have to be investigated.

But then, if you are turning that on you can have a readout across the entire flask of where the hot spots are. If they are stable they would not pick up short transients, like you are discussing, but a stable hot spot over a prolonged period of time that could generate an artifact might be detectable that way. So, that is something we are thinking about pursuing.

DR. MOROS: There are two general points from the engineering point of view on the thermal control point of view. During exposure most people want to keep their cells at 37 degrees. If the SAR is relatively low, so if you imagine this sample where the cells are, imagine them inside

an adiabatic box, a box that does not allow any heat loss. If your SAR is low, then you can expose this sample inside and your temperature increase will be very minimal over time, but it will increase because the box is adiabatic. But if you have a high SAR, then you are going to have a rapid increase. So, the thermal control requirements for low SAR, medium SAR and high SAR change drastically, and the goal is to maintain the cells at 37. However, all cells will experience during the initial period, when they are put into the irradiator, a trajectory because, one, the irradiator may not be at 37 already or, two, the cells may not be at 37 already, or the microwave is turned on and the system needs to equilibrate. But the goal at steady state, if we can call it that, after 20 minutes or so will be to have the cells at 37.

Now, it gets confusing at times because when you are measuring SAR -- measuring SAR -- you want the opposite. You want to increase the temperature as much as you can. So, sometimes these two things get confused. So, I am not going to bring up the second point but the first point is that you want to keep it at 37 and the higher the SAR, then the more problematic it gets.

DR. ROTI ROTI: I want to mention something that came out of Eduardo's remark. We found very early this problem that if the cells weren't at 37 when they were

introduced in the room would cause all kinds of artifacts. So, as a standard operating procedure all cells are cultured 24 hours before the exposure starts. So, they are well equilibrated and the only possibility for any temperature drop is carrying them from the incubator to the RTL room, which is a very short distance, so that we can avoid any artifact from a temperature drop.

DR. HOOK: Well, we have a little experience with human blood, cells in culture and mouse lymphoma cells, and some bacterial cells and we actually found that human blood held its temperature the best and it still dropped a degree every 30 seconds in the transport. So, just that transport from your incubator you have a major temperature drop that is going to happen.

DR. ROTI ROTI: But under our conditions that did not produce measurable biological effects, whereas culturing the cells and starting up the experiment did produce measurable biological perturbations. So, we had a 24 hour pre-culture period in there every time.

DR. OWEN: Good. I think that primes us very well for this afternoon's presentations and the discussion that will follow that. So, we can break now and then when we come back together at one o'clock we will begin with the first of the data presentations.

[Whereupon, at 11:40 a.m. the proceedings were recessed for lunch, to resume at one o'clock]

AFTERNOON PROCEEDINGS

DR. OWEN: Let's try and get rolling. First of all, I would like to remind people to try and use the microphones when they speak, and I had a miniature tutorial which I will try to repeat for you. It is important to be facing the microphone and not be very far away from it. Because of the peculiarities of this room, if the system is turned up very loud we get a lot of feedback. So, you need to be fairly close to the mike when you are saying anything.

Secondly, I have done some brief introductions before people have given talks, but people have requested sort of an all around introduction. So, what I would like to do is to ask people around the table to introduce themselves, starting with Dr. MacGregor, on this end, with these two tables of the working group and then moving right along to the presenters at the third table, please.

DR. MACGREGOR: I am Jim MacGregor. I am Director of the Office of Testing and Research at the Center for Drug Evaluation and Research at the FDA.

MR. BASSEN: Howard Bassen, Chief of the Electrophysics Branch in Office of Science and Technology, FDA.

DR. WILLIAMS: I am Jerry Williams. I am Professor of Oncology at Johns Hopkins Medical School. I have an interest in molecular and solar radiobiology.

DR. VERSCHAEVE: I am Luc Verschaeve. I am a biologist from the Flemish Institute of Technological Research, but I introduced myself a couple of hours ago I think.

DR. LOTZ: I am Greg Lotz, and I am with the National Institute for Occupational Safety and Health. I am Chief of the Non-Ionizing Radiation Section there, and I have a physiology and biophysics background.

DR. OWEN: Russell Owen, Chief of Radiation Biology Branch here, at the Center.

DR. FENECH: Michael Fenech, from the government research institution in Australia, CSRO.

DR. LAGROYE: I am Isabella Lagroye, Assistant Professor in the Bioelectromagnetics Laboratories in France.

DR. ELDER: My name is Joe Elder. I am employed by the U.S. Environmental Protection Agency at the National Health and Environmental Effects Research Laboratory, in Research Triangle Park, North Carolina.

DR. CHOU: C.K. Chou, from Motorola, Florida Research Lab.

DR. ALLEN: I am Jim Allen, research biologist at the EPA, in Research Triangle Park, North Carolina.

DR. TICE: Ray Tice, Senior Vice President for Research and Development at ILS in Research Triangle Park.

DR. HOOK: Graham Hook, and I am Director of the Genetic Toxicology Program at Integrated Laboratory Systems in Research Triangle Park, North Carolina.

DR. ROTI ROTI: Jo Roti Roti. I am in radiation oncology at Washington University and that is in St. Louis, and I am doing some RF hyperthermia and ionizing radiation research.

DR. MOROS: My name is Eduardo Moros. I am from Washington University, Mellincrot Institute of Radiology. My background is in engineering and medical physics.

DR. OWEN: Thank you, all. Now, on our agenda we have a presentation of results from Drs. Tice and Hook.

Presentation of Results

[Slide]

DR. TICE: Graham and I are going to give a two-part presentation. Graham is going to talk about the dosimetry and I am going to talk about the biological effects.

The one thing I would like to mention with a little bit of an apology is that this paper finally did get sent into BEMS just recently, as at the end of last week. This is the data that talks about both the DNA damage and the micronucleus induction, the data that we are going to be

talking about today. I also want to point out that this work was sponsored by Wireless Technology Research.

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I also want to mention that WTR sponsored a variety of in vitro assays to be looked at within the radiofrequency program. We looked at mutations in S. typhimurium and E. coli, mutations in mouse lymphoma cells, chromosomal aberrations in proliferating human lymphocytes, and then this presentation will focus on DNA damage in human blood leukocytes, micronuclei and human blood lymphocytes. I just might mention that all the other assays were negative and will be part of other papers that will be going out sometime between now and, hopefully, October. Graham?

[Slide]

DR. HOOK: As Ray said, I am going to describe the exposure system and, to some extent, focus on how we utilized it. The system that I am going to talk about is the transverse electromagnetic cell exposure system that C.K. described earlier.

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So first some acknowledgements, Carl Sorenson, Dr. C.K. Chou and Don McDougal for basically putting the TEM cell system together for us, and Dr. Guy for doing all of the FDTD measurements that I am going to describe, and Dr. McCree who helped to establish the system, Integrated

Laboratory Systems, and did some of the time temperature profiles for the PCS situation.

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As I said, the exposure facility was established at Integrated Laboratory Systems. It consists of transverse electromagnetic cells in a radiofrequency box that is inside so that we are protecting the outside from anything stray radiofrequency radiation being admitted by the system. It consists of two transverse electromagnetic cells set up in series and operated in a vertical position. Cells are exposed in test tubes with the long axis parallel to the direction of weight propagation. Dr. Chou showed a diagram of the orientation earlier.

To take advantage of the most uniform portion of the SAR distribution, we set it up so that the cells were mixed, 0.6 ml of blood in 1 ml of medium. It was actually 0.6 ml of blood plus 0.4 ml of media to give a total of 1 ml. That was put at the bottom of the tube, and then another 9 ml of media were slowly layered over the top of that.

From our experience in that system, we know that the cells stayed in the bottom 1 ml and, in fact, within the ten minutes or so that it took to set that up and get them into the system, the cells really settled into the bottom one-third to one-half ml of the test tube.

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The temperature was controlled using a circulating water jack, internal fans and a water bath hooked up to the circulating water. The culture temperature was monitored throughout each exposure using fiberoptic probes, and recorded by hooking the Luxtron up to a computer.

The goal during the exposure period was to achieve a temperature of 37 plus/minus 1 degree Celsius. For all the studies that we did, both for the micronucleus SEG studies but also all the others, we were able to maintain that temperature at the area where the cells were. You will see that it did fluctuate in other parts of the tube though.

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The RF signal was input during the exposure. We measured both the input and reflective power using a power meter or a chart recorder for the case of CDMA technologies, but we only recorded the resultant power. However, from our measurements we showed that the reflective power was less than one percent of the total input power.

The signals in each case were amplified using Kalmus amplifier for the 837 MHz situation or Amplifier Research amplifier for the PCS situation. In the case of the analog and TDMA exposures, we also had voice modulation that was supplied by attaching a compact disc to the voice recorder or to the cellular telephone.

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For the RF exposures produced in the analog case by a signal generator instead of 837 MHz and frequency modulation of 12.4 KHz, and that was voice modulated, as I said. The TDMA was a cellular telephone supplied by Motorola, as was the CDMA situation. Both of those were in test mode and in the TDMA it was voice modulated. The CDMA could not be voice modulated because the test mode used did not allow us to do that. The last type of phone we used was a PCS-GSM type. That is a 217 Hz modulation, and again set in test mode. The frequency in this case was 1909.8 MHz.

[Slide]

This is the same diagram that C.K. showed earlier, just showing the SAR distribution throughout the tube.

[Slide]

This is a histogram showing the SAR distribution curve voxel over the whole 10 ml. As you can see, there is a fairly large variation around the mean for 1 W input power.

[Slide]

This is a histogram showing Watts occurring in SAR distribution in the bottom one-third of the 10 ml solution for the 837 MHz situation. Here we have a much more uniform set up.

[Slide]

This is the SAR distribution for the 1909.8 MHz situation, again, 1 W input power -- a lot of difference in the two situations. However, in both cases we did have hot spots that were in the middle of the tube.

[Slide]

SAR distribution for the whole 10 ml solution. A lot of variation over the 10 ml, and again a much more uniform distribution if we look at the bottom one-third ml which is where the cells were.

The other thing to note, these were both for 1 W input power. If you look at the mean SAR, in this case it was approximately 5 as opposed to about 0.4 for the 837. This meant that we had to put a lot lower input power to achieve the same SAR for the PCS situation as opposed to the 837 situation.

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This is just for people's information. Most of this information is in Dr. Guy's paper that was published in BEMS in 1999. For the blood situation, these are the dielectric properties that were used by Dr. Guy to do the XFDTD dosimetry evaluations.

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Again, this was presented earlier by Dr. Chou. This is what we used for our input power settings and the Lauda temperature settings for the different SARs that were

used. I should note that for the PCS situation 2.5 was actually 2.9 and 1 was 1.6. So, those were different but for simplicity I just left it here. Again, to achieve 10 W/kg for PCS we had to use about one-tenth the input power to achieve the same SAR.

Another thing to note is that in the system the two TEM cells were not identical, of course. They all varied because of the ability of the fan to cool down of the Lauda effects. So, the temperature settings were actually different for each one, and these temperatures were set up by Dr. Chou and John McDoogle, out at City of Hope, for us.

[Slide]

Exposure, durations and SARs tested -- we did two sets of studies, one at three hours. In this case, we evaluated two different SARs per time. So, we had three three-hour exposures. In the first the combinations were 10 and 5 W/kg we did together, or we would do 2.5 and 1 W/kg together. Then, the third exposure was always the control. So, we did the positive control and sham exposed controls as the last exposure period. So, we do all of these in one day. So, the controls in this situation were done the same day but were not concurrent with the RF-exposed cells.

For the PCS situation, the combinations were actually 10, and 2.9 and 5 and 1.6 that we did together and, again, the controls at the end.

For the 24-hour situation, because we can only do one SAR at a time because we used the other TEM cell for the positive and controls, so in this case the controls were done concurrently with the RF-exposed cells. So, the SARs that were used in this case for the 837 situation, analog, CDMA and TDMA were 1, 5 and 10, and for PCS we did 1 and 10 W/kg. Because we could only do one SAR, each experiment was independent.

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This is just a representative profile for our three-hour exposure showing the time temperature effect. The temperature of the blood when we started -- when we first got the thermistor probe into the TEM cell ranged from 25-32 degrees Celsius. It took a maximum of 20 minutes from when we started the exposure until the cells reached 36 degrees. So, it ranged from about 5 minutes to 20 minutes before it reached the 36 degree point.

As I said, what we were shooting for was reaching equilibration temperature of about 37 but that did vary up to about 0.5 degrees C. depending on the experiment. We had four probes. The tubes that had the probes in them had blood samples in them. We didn't use those for the experiment but they did actually have blood samples in them. So, in each tube we had two probes, one that we set in the middle where we had seen from the SAR distributions that

there was a hot spot, and one probe would go in the bottom. So, for TEM cell A, in this case being exposed to 10 W/kg, you have about a 0.5 degree difference between what the cells were receiving in the bottom and what was seen in the middle. At 5 W/kg we did not see that effect. In fact, only at 10 W/kg did we really see that temperature difference.

DR. WILLIAMS: The difference between A and B is simply positioning?

DR. HOOK: They are set up in series so there is a TEM cell and then what happens is that we had the input power going into the first TEM cell and that is where we set the input power. So, we set that one for 10, and we had coaxial cable between the first and second TEM cell which attenuated the signal. So, we achieved the second power level that way. So, that is how they are set up. In this case the A TEM cell is always the higher power over the B.

[Slide]

This is a representative temperature profile for 24-hour exposure. I picked PCS so you could see the effect at the middle in this case, and some of the fluctuation that we did see over 24 hours. In this case, over 24 hours we did have much greater fluctuation. A lot of the big fluctuations you have here were actually related to the cooling fans that we had for the entire room. The room was

sealed and we had fans built in there but a lot of heat was generated over time by the amplifier and all the other electronics. So we had cooling fans, and when they would kick in they would actually drop the room temperature down and that did have an effect on the temperature in the TEM cells. But, again, we always had an equilibrium temperature of about 37 and we didn't really see much more than about 0.5 degree fluctuation even over a 24-hour period and, certainly, we never got above or below the 1 degree Celsius that we had set as our goal.

One thing to note here in the PCS situation, the hot spot was actually about a degree higher than you would find in the 837 situation. The other thing to note is that this degree difference was maintained over the whole 24-hour period and we saw this every single time. So, either over 3 hours or 24 hours, this difference in the temperature between the middle and the bottom was maintained. So, there was no equilibrium reached over the whole tube. These hot spots remained as hot spots. There did not seem to be anything circulation of temperature -- circulation in the fluid which cause the uniformity of temperature to develop.

MR. BASSEN: Is this in the center of the test tube or at the edge?

DR. HOOK: It was right on the edge. Actually, because of the way the probes were set, they would usually

flip over and actually almost be touching the plastic of the tube.

MR. BASSEN: Did you measure any gradient within the tube? What is the air temperature outside the test tube?

DR. HOOK: The closest I can get to that is to say it is equal to what the Lauda temperature was set at. So, those were set somewhere lower than 36 degrees. I don't know what the actual air temperature is, but that would be my closest approximation to what it was.

So I don't know what it is in different parts, but I am assuming that we have hit the hot spot where it is supposed to be, so if we moved it somewhere in the middle, we might get one of the lower spots in the SAR distribution.

But, again, we never saw any situation where the temperatures started to come together. They were always about 1 degree apart.

[Slide.]

Just quickly as a summary, here. The input powers that were used were 17, 8.5, 4.25 and 1.7 W for the 837 situation exposures, 1.6 and 0.16 for the 1909 MHz exposures. The power input to the TEM cells varied approximately plus-or-minus 10 percent. We could see this on the power meters. So we would see fluctuations, but they were never greater than 10 percent of what we set it for.

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The equilibrium temperature was 37 plus-or-minus 0.5 degrees C across all experiences and the temperature varied about 0.3 degrees C around this equilibrium of about 0.3 degrees C for a 3-hour exposure and about 0.5 degrees for a 24-hour exposure. So the variation I am talking here is between experiments or between tubes in different experiment, so between TEM cell A or TEM cell B.

That is the end of my presentation.

DR. TICE: Thanks, Graham.

[Slide.]

What we are going to next do is present the biological data that was generated from this exposure system. The parties that were involved in the biology includes Maria Donner, Graham Hook, Marie Vazquez and Daphne Blackburn.

[Slide.]

The protocol was at the end of the exposure period. This is the protocol for the micronucleus assay. Cells were pelleted and resuspended in fresh medium. Phytohemagglutinin or, PHA, was added to stimulate lymphocytes to proliferate. So one of the things to remember is we started with whole blood which is a population of leukocytes. About half of those leukocytes are lymphocytes.

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When you expose them to a mitogen--in this case, PHA--you get a certain proportion of lymphocytes stimulated to divide. So they started off in G-0 and they hit their first metaphase somewhere--starting around 30 hours, a small population can start coming through.

We added cytochalasin B, which is actually a technique developed by Michael Fenech. It was added at 44 hours postexposure or post the addition of PHA to induce binucleate cells. Cultures were terminated at 72 hours and then processed for analysis.

[Slide.]

Just to show you a quick picture. In this case, what they are doing is treating cells. You are getting an acentric fragment during additional cell division. When you look at a binucleate cell, which is up here, you can see a small micronucleus being present. Those are the kinds of objects that we are looking for.

[Slide.]

Micronuclei, as has been mentioned previously, arise from two mechanisms. They have a very basic, very different dose-response curve. One is structural chromosome damage giving rise to an acentric fragment. Another one we call numerical chromosomal damage which gives rise to a lagging chromosome which then gets built into a micronucleus, also.

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[Slide.]

We used two donors in this particular study. Peripheral blood was obtained with informed consent from healthy non-smoking male adults. A single donor was used for each technology. The same donor was used for all analogues, CDMA and TDMA experiments, and I was on travel for the PCS so they took somebody else.

[Slide.]

We had positive and negative controls. They were run the same day for the three-hour experiments, as Graham described, and they were run concurrently for the 24-hour experiment. Negative control was duplicate cultures without an applied RF signal. Positive controls were in the same TEM cell, consisted of EMS, again in duplicate cultures and there were different doses depending on which assay it was run at and the length of the exposure.

In all cases, the positive controls were positive except for one, the first 24-hour study; the EMS dose was too high and it was not scorable. But, in that particular experiment, there was also a positive increase in micronuclei due to the RF signal. So, therefore, that still was an acceptable study.

I might mention that all of these experiments were done under good laboratory practice.

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The endpoints were cell viability. We have a dual dye stain that measures both the integrity of the cell membrane and whether or not the cell, itself, is metabolically competent. We looked at DNA damage in blood leukocytes using the single-cell gel assay at the pH about 13.0. We looked at the frequency of micronucleated binucleate cells, lymphocytes. I also want to note that a single score was used in each study. In fact, for the micronuclei, it was the same study. Coded slides were used, so there was blind scoring.

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I want to mention this because it does bear a little bit on interpretation of the data. We did two kinds of assays with the leukocyte which was a low-molecular-weight diffusion assay. That allows us to look for the presence of necrosis of apoptosis.

And then we did the alkaline pH-13 assay, mostly to detect single-strand in alkali-labile sites where we measured mean tail length for migrated DNA750 and tail moment. We also looked at tail moment H, which is a measure of the variance divided by the mean, or measured dispersion, to let us know whether there was a subpopulation of cells showing increased DNA migration in an otherwise normal population of cells. So we actually had two ways of looking at the data.

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[Slide.]

In the micronucleus portion of the assay, we look at the replicative index which is the relative frequency of cells with one, two, three or four nuclei, to measure the rate of cell division. We also measured a binucleate cell index which is the percentage of binucleate cells per culture. It is more of a stimulation index. And then we looked at the frequency of micronucleated binucleate lymphocytes.

Again it says the number of cells that were scored. We looked at 2,000 cells per each replicate culture.

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The statistical analysis for the toxicity data, which is based on individual culture responses, was a one-tailed trend test and a one-tailed student's t-test where there were multiple SARs tested, which was the 3-hour exposures. When the single SAR was used, it was a one-tailed student's t-test.

For the micronucleus data, we used a one-tailed binomial trend test and a one-tailed Fisher's Exact test to look for increases at multiple doses within that particular experiment or just a one-tailed Fisher's Exact test.

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The toxicity results for the 3-hour exposures; there was no consistent pattern of toxicity except for, with TDMA, there was a significant dose-dependent decrease in the replicative index in replicate experiments with a significant depression 10 W/kg.

In the 24-hour exposure, there was, again, no significant pattern of toxicity except for that the BCI was reduced at 10 W/kg in replicate analogue experiments. So, generally, overall, there was really no sign of great depression in the rate of cell division or in what proportion of cells were stimulated by PHA.

[Slide.]

This is the 3-hour data presented schematically. You can see, up here, that analogue 1 is this dark line right here. At this particular dose, there was a significant elevation in the frequency of micronucleated binucleate cells at that dose only, even though there wasn't a significant trend test.

We decided to replicate it. The next one is the yellow line. You can see, in the yellow line, there was no increase whatsoever. We concluded that, for all technologies tested, there was no increase in the frequency of micronucleated binucleate cells following this particular exposure protocol.

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This is the 24-hour data. I want to point out to you the following. This is at frequency of micronucleated binucleate cells, actually per 1000. The green represents the control frequency. You can see it bounces around a little bit, generally maybe between 0.2 and 0.5 micronucleated cells per 1000.

The 10 W/kg, or the red lines--you can see that it goes, in this case, from about, say, 0.4 up to about 1.4. In most of the cases, whether there was a significant increase, we are talking about a frequency of 1 extra micronucleated cell per thousand in the population.

All the red bars were significant statistically at a p-value of less than 0.001. If you look at the 5 W/kg, and this was the first experiment, every time we got a positive, we replicated it at 10 W. So this is the 2nd, first 2nd, first 2nd.

When we did the second experiment, we did them two days running. I might mention that, because these were 24-hour exposures, and Graham was in charge of the exposures, that meant he was locked up in a little room for almost 48 hours. I think he took a nap between one setup and the next set before he did it. If anything, that probably had more of an adverse health impact than any of the other exposures.

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In any case, we also tested 5 W/kg on the second day running at the second experiment with analogue and also the second experiment with TDMA. You can see this is sort of a dose response, but, in this case, the frequency was pretty much the same. Both of those are significantly different from the control.

When we tested it at 1 W/kg, which was the CDMA and the PCS, there was a small elevation, but it not significant, not statistically significant. And that probably about all we can say about it.

[Slide.]

The comet results, just to point out what they were, is that there was not a significant increase in DNA damage measured either as mean tail moment or as subpopulations of cells with increased tail moment under any exposure condition or any technology, or any SAR, and no toxicity.

[Slide.]

The micronuclei; for 3-hour exposure, there wasn't a statistically significant increase in the frequency of micronucleated binucleate lymphocytes, but, at 24-hours, there was a significant increase that was reproducible across technologies at an SAR of 10, and for two technologies, for an SAR of 5 W/kg.

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The magnitude of the response, which was generally about fourfold or about a 1 micronucleus difference between the control per 1000 cells was dependent upon the exposure duration, positive at 24, negative at 3, and dependent upon the SAR, positive at 5 and 10, negative at 1.

But it was independent of technology, independent of the presence or absence of voice modulation, and independent of the frequency of the SAR signal, 837 versus 1909.9 MHz. Remember, we had two donors and, at no time, did we test the donors at the same time. So it is kind of hard to say anything about donor-to-donor variability. But across technologies, the magnitude of the response was about the same so nothing was appreciably obvious to us there.

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There are two questions that, of course, come to mind about the micronucleus. One if them is--well, let me go down here; what is the mechanistic origin of the induced micronucleus. You might note that there was a lack of a positive comet assay so that might suggest that the increase wasn't due to DNA damage, but that is reaching a little bit.

Is micronucleus induction due to localized hyperthermia, temperatures of 40 degrees Celsius or above, because we know that hyperthermia induces micronuclei. Unfortunately, because of the way this study was designed, there were ways of detecting the origin of micronuclei. The

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most typical way, using an antibody, is to look at the presence or absence of a kinetochore.

If the micronucleus has a kinetochore, it is considered, de facto, that that means that the micronucleus contains an intact chromosome associated with numerical chromosome damage. If it doesn't contain a kinetochore, then it means it is an acentric fragment and, therefore, it refers to structure chromosome damage.

To be able to look for the presence or absence of a kinetochore, you have to fix the cells in a particular way. The way we are doing it in these studies precluded that particular kind of analysis. Basically, we destroyed the epitope by using three-to-one methanol and glacialacetyic acid.

The other way to do it is to go back and try and size them. We talked about doing that, but we thought that was kind of in inferential way of determining the origin and we thought we would put out for trying to look at a more basic understanding using a kinetochore antibody. But that was done within the time constraints that we have for the studies.

[Slide.]

If we talk about future research, the kinds of things that we came up with--and this is conversations between Graham and I and the staff at ILS. It also involves

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other kinds of discussions with colleagues and at other kinds of meetings that we have been to.

But, clearly, reproducibility is the hallmark of response. We think that this should be looked at at other laboratories. For all we know, although we got a positive response with every technology, there might be some consistent pattern that we are doing that gives us that response that that is not meaningful.

We don't think so, or we would like to not think so, but, again, any good science project or any good assay, the end result gets reproduced independently in some other lab to show that it is not just lab-specific.

We think somebody should extend the dose-response relationship. We know it is negative at 1.0, positive at 5.0. Where is the breaking point for that? Extend the exposure-duration relationship; we get negative response at 3.0, positive at 24. Why do we actually go to 24? It is because we are trying to mimic the other in vitro studies we are doing with chromosome aberrations where there is both a short exposure, a limited short exposure, and a more extended exposure.

We were trying to mimic that particular study but, as far as I can tell, we are the only ones that have ever done a 24-hour exposure to RF signals.

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Evaluate the role between modulation and carrier wave because there is both modulation and carrier wave involved in this. Determine the mechanistic basis of the induction of micronuclei looking for the presence of a kinetochore. Because N.P. Singh and his comet studies of rat brains has concluded that the damage there is due to the presence of free radicals, maybe we can look for free radicals in these studies. But, since the comet assay was negative, that one is kind of an "iffy" thing to even bother looking for.

[Slide.]

Determine the possible role of localized hyperthermia in these results because we are talking about the potential interaction with temperature gradients. We came up with three possibilities, some of which have already been mentioned; evaluate the relationship between culture temperature and the induction of micronucleated cells in the absence of an RF signal by controlling the temperature at which the cells are maintained for 24 hours by using an lower average culture temperature.

One of the studies, though, that got an increase in micronuclei exposing blood used a temperature of about 22 to 25 degrees. I guess they were ambient temperatures. And they still saw an increase. But they may not have controlled for localized heat effects.

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Of course Joe has been talking about also, today, and will present in his talk about the ability to look at heat-shock proteins on a cell-by-cell level and that might give us the most information about heat effects within it. Of course, once you put all this data together, clearly somebody has to sit down and talk about the biological relevance of these findings to human health.

Our job was to look at hazard assessment, not to look at risk, and that is what we did. That's it.

DR. OWEN: Thank you.

I would like to go on right now to the presentation by Drs. Roti Roti and Moros and, as we did this morning, then take discussion as a whole after those initial overviews are complete.

Presentation of Results

DR. MOROS: Good afternoon.

[Slide.]

This is an old slide, but the purpose is to remind you that this work requires a team. The members of that team, I am glad to say, are Dr. Pickard, from Washington University, who has more years of experience in this field than I have of age, and Dr. William Straube who is working in my lab for the last fifteen years and to recognize that this was awarded by the agreement between Motorola and Washington University.

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[Slide.]

The aim at the research and development of the irradiator at Washington University had the following objective and constraints. The fields had to be typical cellular phones so all the developments were done with that in mind. We were not looking at any other type of exposure conditions but that of cellular phones.

We needed very large irradiated areas; in other words, a very large number of cells. That was one of the key components of the design parameters or criteria. We needed reproducible SARs, of course. We needed a well-characterized exposure. We needed low exposure to working personnel in and around the lab and for those working directly with the irradiators.

One of the things that we bumped into that was very important was the interference with local cellular telephony. It turns out, as you may well know, that these phones are extremely sensitive and they work at very low powers already. So our leakage had to be well below the noise floor which, I believe, is, if I remember correctly, 120 DBMs. It had to be in a temperature-controlled environment for the cells that were inside the irradiators, and also the irradiators had to be inside a controlled environment, maybe in a very similar way as explained by C.K. Chou.

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All of these have to fit in our available space. We had one room that measured 8' by 8' by 8', and we had to put all of these things in there. Of course, you want something that is easy to use by people that are not engineers but are biology technicians, or biologists and even principal investigators.

[Slide.]

So the preliminary design looked pretty much as the final design after it was tested and let me introduce to you the radiotransmission line. So, what is it? Here we have a side cut-out view of the RTL showing the major components. We have a conical antenna at the center. Then we have a fan for cooling, and we have a thermocouple for measuring temperatures.

The flasks; you can see where they are positioned around the conical antenna. Then we have a microwave absorber that absorbs all the energy that is not absorbed by the flask which is probably most of it.

So the wave is launched from the antenna in vertical polarization. The s-vector goes in the direction, of course. And then you have the magnetic-field vector going into the plane of the screen, here. All three components contribute to the SAR.

The bottom is a quarter-inch aluminum plate which serves not only as a mechanical support but also as a very

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effective thermal homogenizer. The top plate was composite. It was some sort of polyurethane material with two thin sheets of aluminum of both sides. That made it very light weight, very easy to lift up by any person. The bottom plate was about 50 pounds, so that would have been very heavy to lift.

The phone is terminated in a perforated aluminum. So, obviously, the fan for cooling--not the cells, necessarily, but the fan is actually for cooling the phone because the phone absorbs most of the microwave energy. It is not shown in this slide, but there are also strip heaters that may come on or off to raise the temperature of the cells and keep them at 37.

The reason for the heaters is because it is a large room. There is air blowing all over the place, as you can see, so you keep the room temperature about 2 degrees below 37 and then the combination of heat deposited in the foam plus the heat deposited in the aluminum brings up the temperature to 37. We were able to achieve a temperature, at steady state, between 37 plus-or-minus 0.3 degrees.

Also, you have to bear in mind that for the sham RTLs, there is no energy deposited in the foam because there is no microwave. So you needed those strip heaters to bring up the temperature of the cells.

[Slide.]

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Here is one of the RTLs. We have ten RTLs. We have built, I think, fourteen of them so far. You can see the antenna in the center. You can see where the flask--these are T-75 flasks. The foam; you can see some of the very cosmetic-type leakage control here. The room is also sealed with copper tape and stuff like that to minimize leakage outside the room

[Slide.]

This is looking underneath you. You can see that, for a fairly simple system, things get complicated very fast. You have the wires that run the fans. You have the RF and cable lanes. You have the thermocouple wires.

You may see that these RTLs are mounted in a drawer system so they can actually be pulled and then the top plates lift up like a car hood. You can see that some of them are not connected to any RF because they are sham.

[Slide.]

Here is a close look at the design of antenna. I don't want to spend too much time into it, but there is something to say about this design; it is not optimized. We did several trial-and-error, and then, once we found something that worked, we stopped looking.

We do want to, then, have the opportunity in the future to optimize the antenna and the entire RTL, at least

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numerically first and then do some testing in the laboratory.

[Slide.]

This is a closeup of how it looks, the antenna in the RTL. You can see here the flask. Our flask contained 40 ml of media. There is a reason for that. Most of the biologists, at first, wanted to use 15 mls, but we were not getting enough SARs, either for exposure or for SAR measurement. So, after a series of both electromagnetic and numerical analyses, we decided to use 40 mls as a tradeoff that would allow us to get relevant SARs and also would allow us to measure SARs using thermometric and thermographic techniques.

[Slide.]

This is a look outside the warm room that is behind this wall here. You can see the amplifiers. There is a computer system that keeps close attention to the amplifiers output and to the signal generators and, also, to the temperature.

[Slide.]

This is the screen, looking at the temperatures from all the RTLs. There are two temperature sensors for the RTLs. These sensors were calibrated so that they reflect the temperature inside the medium in the flasks. If we have one temperature that goes off the 37 plus-or-minus

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0.5, they turn red so you can see them. It will tell you whether the system is okay or something is going wrong. You can turn the signal by just clicking on the mouse, on these buttons.

By clicking on these buttons, you can actually see the last 24 hours of data, of temperature data, for the RTLs. It gives you global max, an average, a global standard deviation. This was not just for the engineers and physicists working as a way of collecting the data but also it works very nice for the biological stuff. They learn how to use this very quickly.

This system reads the temperatures every 30 seconds and checked for temperature highs or lows every 30 seconds, but only recorded it on the hard disk every 10 minutes.

[Slide.]

This is a look at the last 24 hours of data in RTLs 1 through 5. This only shows 1 degree in the vertical axis and each one of these lines is 1 hour. So you can see that, by just clicking on it, you can get a feeling for whether you had a low temperature in the last 24 hours, or too high of a temperature in the last 24 hours.

The reason there is a number 5 in between 34 and 35 is because it is not measuring RTL. It is measuring the room air temperature.

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[Slide.]

Also, there was a protocol worked out not to avoid excess leakage into the environment. Remember, we were using real signals. They were modulated. We didn't want to interfere with local communication even though we did have a channel that was granted to us by Southwestern Bell. The CDMA signal spreads over a great number of channels, so we have to be careful.

Everyone who wanted to get inside the room had to go through protocol filling out all the things that they did and all of the power levels--we have to turn the things back on. So this was part of our GLP efforts.

[Slide.]

What you see here is the return loss for a series of RTLs over the frequency spectrum of cellular phones. The message here is that all of them were better than -6 Db return loss which means they were fairly efficient in converting most of the electrical energy into wave.

[Slide.]

This shows a typical distribution of the RF field strength around the 16 positions of the 16 flasks. Again, it shows that you get, angularly, a very uniform distribution of energy.

[Slide.]

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Our first initial set of SAR measurements were done with fiberoptic probes. We learned very quickly that we had trouble, that we needed to improve our efforts. These are our measurements using that kind of probe at 836 and 2450, SAR levels at several positions on the bottom of the flask.

The average SAR for those four probes was 0.025 for, I think it was assuming 100 watts--per watt of net input power. This is not the power the amplifier reached but the actual power that has gone into the RTL.

[Slide.]

All of these results were published in this paper that described the RTL. If anyone wants a copy of the paper, I will be glad to take your E-mails or addresses and I will E-mail them or mail them to you, whatever you want.

The message from this paper was that we had 16 plus-or-minus 2.5 mW/k per watt from net watt input power. That was at 836 MHz.

[Slide.]

As I said, we learned very quickly that we had problems measuring SAR use in thermometric techniques. This is a simulation to show you, to explain to you, what the problem is. This is the height within the medium. So we have 5 mm of medium height. Just assume that this is at the center of one of the flasks.

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The true SAR, which, in this case, is the SAR calculated by a one-dimensional seven-layer model, electromagnetic model, is this. So assume that this is the true SAR. If you measure the SAR thermometrically, and it is very accurate, then it should fall very close on top of this SAR which is the numerical value.

So this numerical SAR distribution was an input into a thermal model. Then we measure--so-called measure--SAR, really calculate SAR, using the equation that was introduced at the beginning by Howard Bassen.

What happens is that--this is at 5 seconds, 10 seconds, 15 seconds, 20 seconds. Even at 5 seconds, you can see that you are already measuring an SAR that is higher at low SAR areas and lower at high SAR areas. Another important thing to look at from this figure is that we had a very large SAR gradient within the medium.

[Slide.]

This is just to illustrate. These are for measurement. These are not numerical simulations. The top SAR and the bottom SAR, you can see that it is about a five- to six-fold higher SAR at the top than at the bottom. This is problematic. When, then, you are going to measure SAR using thermometric techniques, we could not use an E-field probe in this situation because half the E-field probe is outside the medium.

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So we are really limited to either numerical simulations to measure SAR or to using thermometric techniques. This large gradient from top to bottom confounds the measurement of SAR and you get what we call very high thermal-conduction errors.

[Slide.]

I think I will skip over this one.

[Slide.]

Anyway, we learned that we have to measure temperatures at least in 5 seconds and we developed a temperature differential probe. We were able, then, using that technique, which has been published already, to measure millicalorie change in temperature over very short times. Using that technique, amp-probe, we did very detailed SAR measurements over the bottom of the flask at 136 and also at 2450.

I don't really want to take a lot of time to go over these numbers unless somebody in particular is interested. This data has also been published.

[Slide.]

You can see here--this is testing symmetric position to see that we have symmetry within the flask.

[Slide.]

If we establish symmetry, we only need to map half of the flask. This shows those measurements. Other things

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that we did are, and this is important, that--remember what I said at the beginning that the system was designed for cellular-phone levels of irradiation. So we are talking around 1 W/kg.

Then, as we were doing our experiments, the need or the desire for higher SAR was expressed to us, and, as engineers, we were charged with looking into this problem. How do we increase the SAR using the RTLs. We have limitations because the absorber foam, if it gets to hot, it may transfer too much energy into the flask and temperature control becomes a problem.

So one of the thing we did was to look into dielectric shimming which is equivalent to raising the cell layer of the bottom plate, which we know, from past studies, by us and by others, that that will raise the cell-layer SAR. So, instead of actually lifting the flask, we put a shim of dielectric material to raise it electromagnetically.

[Slide.]

Here, we will show you the ratios of the SARs measured with a dielectric shimming of alumina versus air shimming, which was just a piece of styrofoam. You can see that we can gain between two- and elevenfold increase in SAR with the same power input. So we are increasing drastically the efficiency of the system with the same power input.

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The conclusion of this study was that there was about a 4.3-fold gain in SAR by using dielectric shimming. That is what I will show you next.

[Slide.]

This slice compares the SAR at the cellular-phone frequency that we have been using and 915, which is an ISM frequency you may know. This is an industry frequency. Anyone can use this frequency. It is open to the public. As you can see, the SAR are very close.

The message here is that, if you don't want to go through the very high expense of leakage-proofing your irradiation equipment, you may want to consider using 915, which is an open frequency.

[Slide.]

I am getting to the end of my talk here. These are simulations using the FDTD method. These are SAR distributions on the cell layer for a 100 W net input into the RTL at 836 MHz. This is when there is no shim. This is the original configuration where the flask is positioned directly on top of the aluminum plate. We get an SAR average of 0.6 plus-or-minus 0.4 W/kg.

If we take that same setup, we just include a 3 mm foam. We basically raise the flask in the air. We get a small increase in the average SAR, as you see here. But look at this. When we actually put the alumina, which is

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the dielectric material, we went from 0.6 or 0.9, whichever you want to look at, to 3.2. So that is very nice.

And the SAR, I may say, I think looks better in this case, at least the high SARs in the center, not at the edge, like in this case.

MR. BASSEN: Is that the top view?

DR. MOROS: No; that is the bottom cell layer. That is the bottom voxel.

MR. BASSEN: That is looking at the surface of the cell, the broad surface of the cell culture dish?

DR. MOROS: This is a numerical simulation. The value of the media and the flasks and the antenna and the RTL is all discretized. This is the distribution of the voxels that are on contact with the plastic bottom of the flask. So the voxels are much larger than a cell, than a biological cell.

MR. BASSEN: I am just saying, what are we looking at? Which way--

DR. OWEN: The culture surface. It is like a picture of the culture surface.

DR. ROTI ROTI: That is the surface where the cells are. We are just saying the voxels are bigger than that.

MR. BASSEN: I am just wondering what you are viewing, what perspective; side, top, of the culture dish?

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DR. MOROS: For your orientation, the wave will come in from left to right, and the electric field is going into the plane of the screen.

MR. BASSEN: Which way is the flask--

DR. MOROS: This is the bottom of the flask. This represents the bottom of the flask.

MR. BASSEN: Okay.

DR. MOROS: Is that clear, now?

MR. BASSEN: Yes.

DR. MOROS: Okay.

[Slide.]

We have done similar simulations at other frequencies. These are 2450. You can see the difference between--we have basically the same result that was shown before. When you go to higher frequencies, you find out that you don't need as much power to get the same SAR.

We also wanted to see whether you gain by using aluminum shims. You can see we go from 16 to 25. So we still gain but we don't know, at this point, whether the gain is worth it because the SAR seems to get--it is already nonuniform and it seems to get even more nonuniform at these high frequencies.

We did the simulation over the spectrum from 500 to 3000 MHz. This is the ratio SAR. This is the SAR with the dielectric loading divided by the average SAR without

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dielectric loading, just raising the flask 3 mm off the plate. It shows that the maximum gain is about 15-fold and it is, of course, around 1100 MHz.

But it also shows that, at all frequency, we get some value about 2 at the high frequencies. So even 100 percent increase in SAR is possible. One of the things that we would like to do in the future, as a physicist working in this field, is to optimize the RTLs and the shim, the shim width and the shim material.

As I said, the final solution; we stopped looking because of our constraints. But there is the possibility that there are other materials out there that may not only give us higher SAR for the same input power but also more uniform SAR.

[Slide.]

So, in conclusion, alumina shimming increases SAR at the cell layer by a factor of between 1.3 and 15, as I just showed you. This factor depends on the frequency of the signal. Around 850 MHz, we have a factor of 6; at 1600 MHz, a factor of about 3; and, at 1450; no, 2450--I'm sorry; there is an error here--it is about 1.5.

Shimming is a very cost-effective way to increase SAR. It is very, very cheap. In comparison with trying to develop a better irradiator, it is orders of magnitude cheaper.

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[Slide.]

As the frequency of the input signal increases, the SAR distribution becomes more and more uniform. I think this was shown also by the slides that C.K. and the previous group showed. It is just a matter of the wavelength being shorter. The increase in nonuniformity can be attributed to increases in the scattering reflection and standard wave patterns.

I am not going to talk about this. I don't know if Joe is going to touch on this, but we are one of the groups that argued about the biological impact of nonuniform SAR can be assessed theoretically using the voxel theorem which is a voxel that had been postulated by Dr. Pickard and, I believe, the paper is in press right now. But he had presented it at meetings.

[Slide.]

Accurate thermal estimation on nonuniform SAR in a small volume is difficult due to heat-conduction errors. It may be possible to estimate the heat conduction contribution to the SAR using modeling. At present, I feel that we must rely on FDTD modeling for a more complete dosimetric picture.

[Slide.]

The conclusions are basically that we achieved the design requirements that I showed you right at the

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beginning. We have a large-area irradiation facility. We have 19 RTLs. Each one of them can have 16 T-75 flasks. Currently--I don't know currently, but we have three FMCWs, three CDMAs and three controls.

We have used other signals. We are using 2450. I believe we have done two. And other signals. So, I will give you an impression that we can change the signal and recalibrate the system. But recalibration is needed and important after every SAR or signal change.

Individual chill irradiators house--chill the room. We have no interference reported ever since we started and we have no complaints from the FTC ever since we started. Control and monitor the thermal environment; we are better than 37 plus-or-minus 0.3.

We get adequate SAR levels at the cellular-phone frequency range. And robust, easy and relatively fast, easy to use equipment by non-engineering personnel. Flask replacement time takes approximately three minutes; for all RTLs, approximately 30 minutes. So it is something that is very practical.

I believe that is my last slide.

DR. ROTI ROTI: Just to continue this, now, I will talk about--

[Slide.]

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I will mostly talk about the micronucleus work and I will touch on a few other studies that relate to the micronucleus study. We don't need to repeat the signals.

[Slide.]

The only reason for showing this is we did run the shams concurrently with the two exposed samples so, in all of our studies, shams and signals were run concurrently.

[Slide.]

We talked about the temperature profiles. This shows the thermometry, our engineering approach to measuring temperature. We have been working on a biological approach to measuring temperature and I would like to discuss that first.

[Slide.]

We tried to use the heat-shock response as a way to--well, actually, it started out as a two-fold project and it became a single project toward the end. The first goal was to see if the RF irradiation induced a heat shock or a stress response. Since we found it appears not to reduce the stress response, we are going to attempt to use the stress response as an internal thermometer.

This is what the data--this is the approach. This is how the HSP70 gene gets turned on. The HSF is monomer in the cytoplasm. That is the heat-shock factor which initiates the transcription of the gene. As it is unfolded,

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it trimerizes and binds to the DNA. This is the active form.

[Slide.]

What we assayed for is the activation of the heat-shock factor. If you make a cellular extract and you have an active transcription factor, it will bind with a probe that contains the heat-shock element. So, then, the heat-shock element will be bound to the probe. If it is bound, it will migrate differently in the gel.

This is the unbound probe here. The bound probe will migrate slowly. This is called a gel-shift assay.

[Slide.]

It looks like this in real life. This is a control. These are cells that have not been heated. H is cells that have been heated for fifteen minutes at 38 degrees. That is a 1-degree temperature shift. These numbers here are a dilution, so this means only 5 percent of the cells or 5 percent of the extract is from heated cells.

So we can see that we can see a population that has been heated--if we had a population of cells that was heated to a degree above normal for fifteen minutes and only 5 percent of the cells were in that hot spot, we would pick up the activation of the heat-shock factor.

Did I make that clear? Hopefully, I did.

[Slide.]

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So then we looked at the 5 W/kg experiment. I only brought those. Those are actually Dr. Laslow's work. He has done this with the low SARs, but this is all--for the FM signal, it is 5.1 W/kg and for the CDMA signal, it is 4.8 W/kg. This is five minutes and fifteen minutes. Here is the positive control. No activation of the heat-shock response.

[Slide.]

Here is 30 minutes, 60 minutes and 24 hours. Again, no activation of the heat-shock. This little bit of blackness here is a loading artifact. You can see this for the non-active binding right there.

So I think we can use the heat-shock-factor activation as a way to look for hot spots in any culture that may be showing an RF effect that might be considered a possible thermal artifact when we start getting up to these high SARs.

[Slide.]

We don't really need to show this Dr. Nucleus picture. Just this one looks like a little devil, so I put it in.

[Slide.]

Our experimental plan was to use mouse embryonic fibroblasts. That is because we wanted to keep this in the context of our other studies, which I will summarize toward

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the end. But, because we used the C3H 10T1/2 system for in vitro neoplastic transformation, we decided to collect as much RF data on this cell line as we could throughout our studies.

These were either exponentially growing on in plateau phase. We used the CDMA signal and the FM signal. We exposed for 3, 8, 16 or 24 hours and our positive control is a little bit different from what Ray and Graham used. We used the gamma rays.

[Slide.]

We used the same type of cytochalasin-B blocked cells. We scored 1000 binucleated cells. We scored the data both as micronucleus per 100 binucleated cells and the number of micronuclei per binucleated cell. So we scored that both ways.

[Slide.]

This is our optimization of the cytochalasin-B treatment. We found that for the C3H 10T1/2 cell system--actually it is Kim Bischt, whose name was on the first slide. Anyway, he found that 22 hours was the appropriate block time and we ended up using 2 micrograms per ml, or 22 hours.

[Slide.]

This is the irradiation dose response curve. For exponentially growing cells, it is the top cell. Plateau-

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phase cells is the bottom panel. We have approximately 0.3 Gy as the significant difference for exponentially grown cells and 0.6 Gy for plateau-phase cells. These differences are the student's t-test.

[Slide.]

This is the experimental protocol for exponentially growing cells. These are the exposure times in the RTL. They were taken out and put in cytochalasin B for 22 hours and harvested.

The plateau-phase cells were subcultured after trypsinization to let them go through the cell cycle and we kept them in the incubator for 18 hours prior to the 22-hour cytochalasin-B block.

[Slide.]

This is what the data looks like. We have a sham, 3 W for the CDMA and FDMA. This is at 3 hours and 8 hours. Here is 4.8 and 5.1 W.

[Slide.]

We saw what looked like a depression in this set of data, so we repeated it. It was not significant and the significance of the student's t-test disappeared with further repeats.

[Slide.]

Again, with exponentially growing cells exposed for 16 hours and 24 hours to 3 and approximately 5 W/kg,

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there is not statistically significant difference in any of these. I should have pointed out, these have a slightly higher background micronucleus frequency than do the lymphocytes. That is true for the lymphocyte studies we have done.

[Slide.]

This is during plateau phase, a 3-hour exposure and an 8-hour exposure and there were no significant differences in either signal.

[Slide.]

If we look at 16 hours, again, there were no differences but at 24 hours we found the CDMA signal showed a significant difference--this is the micronucleus per 100 binucleated cells and percent of binucleated cells with micronuclei were both elevated and both statistically significantly different from the sham.

This shows the first three repeats.

[Slide.]

This shows the additional six repeats and it stayed significant in both parameters. However, I would point out, that this is an extremely small absolute increase in percent binucleated cells. It is very small and it is kind of surprising that it got to be significant.

[Slide.]

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This is the FM signal. We repeated it again, and we still didn't get a significant difference statistically. But there is a larger value.

[Slide.]

So, our conclusions for the micronucleus study are that there is no significant increase in micronuclei detected for exposure of cells, exponentially growing cells, in any of the conditions, and also, for the first 16 hours in plateau-phase cells. However, at 24 hours, there was not a difference at 3 W/kg, which is consistent with the previous results that were presented in the previous talks. However, at 24 hours, there was a--well, this is not statistically significant for the FM, but the CDMA signal was significant.

[Slide.]

I think that if we did this study all by itself, we might say, "Well, there is no RF effect." But being aware of the WTR data and knowing what we know about the SAR values that we have and the SAR values that that team has, they have been able to go up to 10 W/kg. We have pushed our system to get to 5 W. We may still have a lot of our cells at a slightly lower SAR, so we may have gotten significant if we had optimized the RTLs. So it is something I think we need to look into in the future.

[Slide.]

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This is with the higher SAR values. This is the comet moment and comet length for plateau-phase cells. This is the irradiation/dose-response curve. I apologize for it being broken. It is a long story why that happened in terms of the graphic design, but there is no detectable comet moment and comet length in either exponentially growing cells or plateau-phase C3H 10T1/2 cells at these higher SARs.

Those of you who might be familiar with our earlier papers, the earlier paper was all done at 0.6 W/kg. So this is now at 3 W/kg and 5 W/kg.

[Slide.]

This shows that, with or without PK in the comet assay didn't make any difference.

[Slide.]

Now, we are a little bit ahead of schedule so I am going to summarize for this group our completed projects that have been ongoing over the last three years.

[Slide.]

We have looked at the growth of the 9L-rat brain tumor in situ, and there are no differences in terms of time-to-death, tumor incidence per number of cells injected and brain waive at the time of death. This is measured with both of these two signals unless otherwise specified.

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This is a 2450 study. We have looked at DNA damage in the rat brain cells by the comet assay and Isabelle Lagroye did this particular study when she was with us to actually compare side-by-side the Olive comet method with the Singh-Ray Tice comet method.

We did each with and without PK and we found no effects in any of these things for DNA damage.

[Slide.]

These are our cell-proliferation studies. We looked at thymidine, uridine and amino-acid incorporation. We looked at cell-cycle progression through s-phase, through s-phase and g2 phases--these are all done with BUDR pulse-chase assays--progression out g2 phase, progression out of g1 phase, progression of g1 cells into and through s. There are no differences found in any of those.

[Slide.]

The reason we designed the RTLs the way we did is so we could do this study which is neoplastic transformation. We picked a seven-day exposure for the first part. It turns out I have one in my brief case, but I don't want to stop talking. We also did 4.5 Gy followed by a 42-day exposure.

So, actually, if you have been keeping track, the exposure ranges that we have studied in this have ranged from five minutes to 42 days. That is another strength, I

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think, of the RTL system is that you can do long-term exposures. In fact, most studies, you will see, are 1 to 4 days, for most of our things.

The heat-shock transcription-factor activation; we have talked about that. These are cytoskeletal and nuclear-matrix protein composition. This was only done at 0.6 W. We probably ought to revisit this in terms of looking at micronucleus effects because, if you are altering something to do with chromatin and not damaging DNA, perhaps, you are altering the way that proteins associated with DNA are interacting with it.

[Slide.]

These are all the DNA-damage studies we have done. Those of you who are on your toes will notice these bottom ones right there. That is a repeat of the Phillips examination with no differences detected. We also looked at apoptosis in those studies and found no difference in apoptosis.

[Slide.]

The micronucleus experiment I just showed you, we found only the CDMA as a significant difference. We did this study with Vijay and we found no differences. It also shows chromosome aberrations.

[Slide.]

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These are some oncogene, protooncogene, expression studies. I should mention that the c-Fos is the first oncogene turned on followed by c-Jun and c-Myc. c-Jun and c-Fos combined to be AP1. And these are other transcription-factor bindings. So this is a pretty complicated study but they are all sort of interrelated.

The interesting thing, as you go through this, is you find positive differences in all of the c-Fox measurements but not in the subsequent c-Jun and c-Myc or transcription-factor activation.

[Slide.]

So we may have a situation, and this is the rest of the studies. These are the original observations with RT-PCR which have been published. The studies with the Northern blots are follow-up studies to see if the effect was reproducible and it is by another method. But the interesting thing about this is that, although the c-Fos levels were perturbed, the subsequent genes did not appear to be affected. So we may not have an effect on the total response, but maybe one of the gene levels is affected.

What that means is an interesting question.

[Slide.]

This just shows you some of the follow-up data that confirms the original observation.

[Slide.]

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This again is looking--now, the interesting thing--I should back up and point out that in exponentially growing cells, there was no RF effect on c-Fos levels. It is only when the cells were in plateau or went from exponential phase into plateau phase that these differences were seen. I should point out, these are all four-day exposures.

[Slide.]

The last experiment; people have mentioned free radicals in at least two talks, so we specifically designed an experiment to look to see if fields interact with free radicals. We decided to do this by using a stimulated macrophage system in which the cells are stimulated to produce oxidated stress by interferon gamma and lipopolysaccharide.

These are the results. You will see we got some positive differences. I didn't bring the entire study with me to look at that, but I would just like to point out that the prooxidant levels in measurable oxidative damage, although this is not a complete measure of that, didn't show statistically significant differences and most of the antioxidant system did not show significant differences. But manganese superoxide dismutase activity did show a significant difference and it was reflected by a loss of viability.

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[Slide.]

This is the way the experiment went. We stimulated the macrophages--this is actually done by Doug Spitz, and I should have mentioned the protooncogene studies were done by Prabat Goswami.

This is interferon gamma stimulated with L-arginine which allows the cells to prime the nitric-oxide synthase which means they will make nitric oxide when the LPS is added.

This is adding L-Nio which is an inhibitory analogue of L-arginine. These cells will not make nitric oxide. So when you stimulate them, you don't get as much nitric oxide produced. Then they are put into the fields.

[Slide.]

I brought a sample of the data. This shows the measured nitrate production which shows that the nitrite is produced. This is the product of it being detoxified and is reduced in the case of the inhibitors.

This is looking at total prooxidants. Again, the fields did not produce any differences in the prooxidant levels.

[Slide.]

If we look at this panel first, you can see the MnSOD is lower. Again, this is a noisy experiment so we can discuss that, if you wish. This is the trypan-blue positive

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cells showing that--actually, it is 1 minus that, but it doesn't matter. It shows a loss of viability coupled with the reduction in antioxidant enzyme availability.

[Slide.]

So what does the global meta-analysis of our study show us? Out of 250 parameters, 13, which is awfully close to 5 percent, showed significant differences from sham. Notice, I have avoided calling these things "effects." I am calling them significant differences from sham.

Okay; 5 percent. If we are looking at 95, most of the Gaussian statistics test for 5 percent differences. Well, you would expect 5 percent of these numbers to be different, so that is an interesting coincidence. However, there are a couple of issues.

These differences are not independent and random and they were all reproduced in subsequent experiments. Eleven of those changes were in c-Fos. Two were in changes in MnSOD. And then the one was in micronuclei.

[Slide.]

So we found, in our global studies, no direct evidence for a carcinogenic effect. The reproducible differences were in C-fos expression, induced MnSOD activity in a stimulated macrophage system--so we would be specific about that--and in micronucleus induction in the plateau-phase cells.

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[Slide.]

I believe that to show these reproducible differences are, in fact, robust effects of RF exposure, we need an SAR dose response. That is why it is so important. We can easily do the first dose--oh, by the way, those last two positive differences were detected at 0.6 W/kg. I believe we can easily do 2.4 W/kg and reproduce these studies. We would like to get the opportunity to do them at 2.4 and 5.0.

Also, I think if there is a dose response, we should extend this to other biological systems and we should have a suggestion of a biophysical mechanism for these before we can actually make a conclusion that those are truly effects, and some evidence for this effect. Anyone can do some pie-in-the-sky speculation.

[Slide.]

Why is that important? I believe that this could be any effect. You don't have to write down the ones we happen to find any difference. These are the ones we happen to find. Somebody else could find something else. But if any of those are real biological effects, I think we can make a better characterization and a better testing protocol to assess the safety of RF signals. So I think that is the main motivation for getting a better handle on what is a robust radiofrequency effect.

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If there is no effect, then I guess what we have been doing so far is fine. If there is an effect, then we at least need to think about have we done the right in vitro and in vivo tests.

Thank you.

DR. OWEN: Thanks, Joe.

I think, because we are close enough to our scheduled break in the agenda, rather than get right into the heat of discussion and then have to take a break, I am going to go ahead and take a fifteen-minute break now and then convene for the discussion.

[Break.]

Discussion

DR. OWEN: I think we got off to a good run this morning before our mid-day break discussing issues that had come up from the morning talks. So I would like to start off now the same way with discussion around the table of this afternoon's presentations.

Come tomorrow morning, we will try and draw together the points, the various topics that are raised in all of today's discussions to get targeted discussion on things that have already been raised.

Would anyone in the group like to kick off?

DR. MacGREGOR: I would just like to raise an issue of clarification with regard to the exposure versus

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expression and measurement. I believe I understand the experiments that were done at ILS where the treatment was done during the exposure period and then the cytochalasin culture went in in the absence of exposure.

But I wasn't clear, in terms of the other set of experiments when the exposure was during exponential growth, they are growing cells, and then cytochalasin is added. Was there exposure during the entire experimental period or was exposure stopped at the time cytochalasin was added.

DR. ROTI ROTI: The cytochalasin B was added after the end of the exposure.

DR. MacGREGOR: After the end; okay.

DR. ROTI ROTI: So it was either 3 hours or 24 hours prior to the start of the cytochalasin B. So the flasks came out of RTL and then cytochalasin B was added.

DR. MacGREGOR: So then I would guess I would follow that up to ask, with that kind of experimental design, then, any micronucleus that you are measuring have to be derived from a persistent lesion that is present that is going to generate a break or disrupt the spindle attachment, or something, during the replication.

I am wondering if others have done experiments where the exposure has been carried out during that period of actual replication and chromosome segregation.

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DR. ROTI ROTI: The only micronucleus we would have lost would have been the ones that were expressed prior to the end of the exposure. So something that actually was generated during the exposure, we might have missed because we only looked at those that were expressed afterwards.

But it is just basically the way we did it. In the plateau-phase cells, they are not going through the cell cycle so no cells are getting into mitosis--except for the small growth fraction that is always present. No cells are really getting into mitosis. It is only in the exponentially growing cells that there is a constant fraction of cells going into mitosis.

DR. MacGREGOR: Right. In the lymphocytes, if I understand it right, was the mitogen in prior to exposure or after?

DR. ROTI ROTI: It was after.

DR. MacGREGOR: So, therefore, in the plateau phase, and in the human-lymphocyte phase, you are exposing nonreplicating, nondividing, cells.

DR. ROTI ROTI: Correct.

DR. MacGREGOR: In the exponential phase, you are exposing cells that are going through that division process but then you stop, and then they go on into a kind of different phase where you are trapping, then, the binucleates. So they are slightly different. But, in both

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cases, you are not continuing the exposure during the period when the cells are coming through into that final measurement phase.

DR. ROTI ROTI: Yes; that is exactly right because that would alter your optimization parameters.

DR. TICE: Although you could do the cytochalasin B during the proliferating-cell phase. You wouldn't do it during the quiescent one because the cells are not dividing anyway.

DR. ROTI ROTI: Right.

DR. TICE: In terms of experimental protocol design, the quiescent cells that Joe did and what we did with lymphocytes are the most directly comparable in the sense that the cells were exposed for 24 hours in a non-dividing cell stage, then stimulated to divide either by PHA or by, basically, subculture.

DR. TICE: Ours were stimulated for 18 hours prior to that, for 18 hours prior to the addition of cytochalasin B.

DR. MacGREGOR: Has anyone done experiments where cells, in fact, have been exposed through the entire period up to the expression and formation of the micronuclei?

DR. TICE: Some of the data that Luc talked about, there is a V-79 setup where they got increases in micronuclei aberrations in V-79 cells that were exposed.

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They were during the exposure but, in a sense, the concern, there, of course, is that there might have been obvious thermal effects because they saw both aberrations and micronuclei.

We didn't do a micronucleus study, but we did an aberration study with proliferating stimulated lymphocytes where we did them for 3 or 20 hours, starting at 48 hours after PHA addition and so no increase in aberrations. But, in that protocol, we did not try and look for micronuclei.

So the exact experiment you are talking about has not been done under the conditions where we have done the other ones.

DR. WILLIAMS: It seems we are in something of an experiment conundrum. In other words, it seems to me the basic hypothesis is that there may be thermal effects or there might be athermal effects. If the basis of in vitro and in vivo toxicity is the dose-response curve, then we would like to separate those.

And yet, it seems to me, that if we try to drive the athermal effects to high doses, thermal effects kick in and either mask it or are the driving force. On the experimental side, if I read the charts correctly, in looking at the voxel distribution, within the experiments, you have a factor of 4 difference in the least exposed to the most exposed.

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I don't know if I read that correctly, in those voxel histograms, the ones on the left were about 2 and the ones on the right were about 8. So, in the absence of being able to separate those out with RF, it puts, really, the impetus on using heat as a tool to try to get a very precise thermal effect curve; time, temperature integrals for the production of different types of micronuclei and other aberrations as well.

But it is really a conundrum which I don't see there is an immediate open solution to how to uncouple the thermal and athermal effects, if they can be uncoupled. They may not be able to be. So maybe we could think along those terms. Is there any way to do that? Is there any way to get a very narrow window on exposure levels and to be able to vary, at that point, whether you can say whether it is--it seems to me that, in many of the experiments, you can say we have multiple populations of cells here.

Some are being heated more than others. Are the heated ones the ones that are contributing to the overall effect? When you do something like micronuclei or chromosome aberrations where you are looking at a relatively low frequency event, then it is more difficult to do. If you are doing induction of heat-shock proteins, then you can do flow-cytometry and see if there is a subpopulation and whether you are affecting that.

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But, in my mind, just in general sense, being, in a way, an outsider, I think that is the overall experimental problem.

DR. TICE: Joe and I have talked a fair amount about that from the standpoint that, again, for us, the thing that we come up with most likely helping to explain the information as you and I have talked about is to, first of all--well, in a sense, they are run concurrently because it is weight of evidence. But you run--it doesn't matter if it is 10T1/2 cells or it is whole blood, but you run whole blood for 24 hours at different temperatures; right around where you think the temperature gradients are, you measure the frequency of micronuclei.

At the same time, you do a replicate RF exposure where you do heat-shock protein levels to see whether or not you got heat-shock protein. Because, if you have no heat-shock protein but you are still getting micronuclei, then, theoretically, that would say that it is not a heat-shock effect.

DR. WILLIAMS: It just says that you can uncouple the heat of aberrations in the induction of--

DR. TICE: The third one is you do the cultures at 30 degrees, or 25 degrees. If you still get micronuclei at that, then that also would suggest it is not a heating--

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DR. WILLIAMS: There is no question that, between room temperature and 37, there is a lot of very sophisticated DNA biology. We did some studies in which we were looking at intercolation in the double helix. There is a huge difference between about 27 up to 37, a big, big effect. So, clearly, at less than 37 degrees, there are changes in the structure of DNA that may be playing a role.

DR. TICE: Which is why you don't just rely on one set of data.

DR. WILLIAMS: I might just say about repeating experiments, that if you do an experiment twice, you are only increasing the power by 30 percent. So the square root of 2.0 is not very big.

DR. HOOK: But, with any mechanism you are talking about having to do with changes due to changing from room temperature to any other, you should remember that all of these studies have controls which went through the same kind of fluctuations as are treated. So the best you could argue is that RF is enhancing some effect because we see a difference between our controls and our treated.

So all these other little side issues, I think, are covered. We have got a control.

DR. WILLIAMS: I am not sure. If there is some synergism between--

DR. HOOK: That is what I am saying.

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DR. WILLIAMS: That's right; you can never separate those out.

DR. HOOK: You can say there was synergism, but you can't say RF had no effect. You can say its effect is synergistic. That would be the least you could argue.

DR. MOROS: I think that what Dr. Williams is saying--with my words, I would say that the effect, the dose-dependent effect that was shown--what you are saying is that you don't know whether they are dose-dependent on the SAR dose or the thermal dose.

DR. WILLIAMS: Yes. And you have a variation in 4, if I think I interpret, in SAR dose within a single flask, or even more. And I wanted to ask, if you change the SAR--for instance, if you have an SAR, an average of 10, is the variation from low to high the same as it would be if you had an SAR of 1, the variation between low and high?

DR. MOROS: Yes; the standard deviation would stay the same, will scale linearly, unless you start to saturate the amplifier.

DR. ROTI ROTI: Something that will be part of whatever we propose to do would be monitoring HSF activation.

[Slide.]

I would like to distinguish between HS activation and the heat-shock protein expression. The HS activation is

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just activating the transcription factor. The subsequent gene expression comes later, and we don't know how sensitive that is yet.

We do know that HSF activation can detect 5 percent of the cells heated to a degree for fifteen minutes. We don't know if we can do better than that and I think we can do better than that.

But, at the same time, Dr. Hunt in our group has just completed linking the GFP to the HSP-70. Now, murine cells express no HSP-70 unless they have been heated. We don't know how sensitive this will be, but they are going to create stable transfectants.

We also don't know if the GFP can be fixed. Assuming the GFP could be fixed in situ after exposure, you could actually do the very experiment that Dr. Williams is talking about by correlating the presence of the micronuclei with the amount of GFP that is present in the cell.

But the issue is going to be sensitivity.

DR. WILLIAMS: But you feel fairly strongly that the thermal effects of micronuclei would be coupled to the induction of HSP-70?

DR. ROTI ROTI: If the cells were heated, the HSP-70 should be induced, assuming it is sensitive enough. And then you can then correlate the presence of the micronuclei with subsets of that population. You could actually sort

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those. The trouble is, they would be binucleated. But you could, theoretically, sort them by GFP and score a thousand from each sort bin and see where your micronuclei were.

DR. ALLEN: I would like to add one thing that I think could be of interest in this. It is known that heavy expression of HSP-70 does protect from chemical induction of micronuclei.

DR. ROTI ROTI: Right.

DR. WILLIAMS: So this could confound the interpretations of the micronuclei that you are trying to correlate with the HSP-70 expression levels.

DR. ROTI ROTI: Right.

DR. WILLIAMS: It may be difficult to make those connections.

DR. ROTI ROTI: But at least we would know they were hot spots that were hot enough to cause the cells to express the HSP-70. If you didn't find--you could actually do the negative. You could sort the cells that had no HSP-70 and then score the micronuclei in those. Then you could exclude cells that were known to be heated, so you can actually use that as a negative condition.

MR. BASSEN: I think to uncouple temperature effects from electric field or SAR effects, you simply would do what has been suggested, is do the experiment twice or several times at an ambient of a half a degree lower and

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higher than 37. That way, the absolute temperature is shifted, but the temperature elevation would occur in the experiments.

I don't think there is anything magic about the temperature elevation and the SAR being distinct. I think you have to do it at two separate levels to separate those two.

DR. HOOK: I guess I would like some explanation on that because I just can't correlate what we are doing with what people are talking about as thermal effects.

We measured the temperature in these flasks, or in the tubes. It was that 36.5 and 37.5 degrees C. Admittedly, that is some average temperature where we had the probe. But there is a measure of temperature. Now, people are saying that my data could still be explained by a thermal effect. So, dropping the temperature by half a degree, measured in my thermistor probe, I don't see why that removes that argument because they are saying that my measurement has no relationship to what they are saying is a thermal effect.

So, just some explanation.

DR. WILLIAMS: Joe, is there a good temperature-response curve for the induction of micronuclei?

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DR. TICE: I am not aware of it. It might be in the hyperthermia literature. I should go back and look for it.

MR. BASSEN: Wouldn't you get that with your shams for the experiment that I proposed?

DR. ROTI ROTI: Yes; we could do that. I suspect that Jerry's question is that the people who looked at micronuclei after hyperthermia tended to look at therapeutic temperatures because they were using it for trying to treat tumors. So I don't know that there is going to be a lot of low temperature sensitivity for microwave effects.

I just don't know that that is there. It wasn't until I actually started working on RF that I became concerned with pushing these assays to their lower limits, for obvious reasons. I think that is an important part of any of these studies, is there a strategy to push the positive controls to their lowest limits.

MR. BASSEN: Until you do a study where you vary the temperature, you are always going to have detractors that will say, "This is a thermal effect." They always say that about microwave. Whether it is or isn't is irrelevant if it occurs at two different ambient temperatures for the same dose.

DR. LOTZ: Howard, in that regard, isn't most of the suggestion--and Graham, this goes back to what you just

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said a minute ago--you have still got a thermal effect on the rationale that, because of the inhomogeneity in your tube, presumably you have some cells which are exposed to a much higher temperature than you are able to detect.

I think that is the rationale. And the question is how important is that. What proportion is it? Is it a significant enough proportion to really bias the finding that you have or is the fact that that is a small proportion why you have a small effect and if you had them all exposed to that higher level, you would have a much more robust effect.

DR. TICE: But getting back to what Howard was saying also if, at 37 degrees, I don't know what the increase in temperature is as you go out to where that high SAR is. But let's just say that hyperthermia normally is said to be occurring at 40 degrees. So let's say that we have got a small population of cells at 40 degrees in a few voxels somewhere scattered throughout that 1 ml, or one-third of a ml, so that is 37 to 40. That is a three degree increase.

If we do the cultures at 33 degrees, or 34 degrees, we are still only going to get a 3-degree increase, I am assuming, with the distribution. In that case, if you get micronuclei, then it is not hyperthermia. If you don't get micronuclei, the question that Jerry was

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saying is that maybe the cells are different at 33 degrees than they are at 37 and, therefore, you have lost the ability to get response.

So you are sort of caught both ways.

DR. WILLIAMS: The variation within the culture dish from exposure is not particularly inherent to the type of exposure apparatus scale but simply on the wavelength of the irradiation?

DR. MOROS: No, no. It has to do it with everything. It has to do with the wave length and the design of the apparatus.

DR. WILLIAMS: No; I am saying, if you built a huge expensive piece of apparatus where this is sitting in the middle of that, that would not solve all the problems.

DR. TICE: I would point out one thing that we didn't mention, and you might reflect back on, is we get the same magnitude of response regardless of whether it is TDMA, CDMA, analogue or PCS. Now, three of those are 837 MHz, but PCS is at 1909.8. The variation around that was much greater. There was much more variability in temperature in SARs, and also temperatures, because of the hot spot, than there was for the 837.

But, again, even though those conditions are different, the magnitude of response was the same which is another way of saying to me that maybe it is not

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temperature, because I wouldn't expect them to give us identical results.

DR. HOOK: And there is data where we didn't see responses, Joe's data, Marty Meltz did some work, too, all of which were in situations where there were temperature increases. Marty Meltz--he is not here to present his data, but he did distributions on his and his SAR distribution was greater than ours. And so he had higher values than we saw and he saw no effect.

So if it is just temperature, we are just not seeing it. So there is something else there. I think what I am saying is we have got a lot of data here and you could probably parse out a little bit of the answers as to whether or not there is some kind of point SAR effect that is leading to these data.

DR. WILLIAMS: May I ask another question? The variation in an SAR in the culture vessel, does that also imply that there are variations in the relationship of the magnetic, the electric, component, that there are changes in the nature of the waves at any of those points because of interference or anything of that nature?

DR. CHOU: The SARs, you saw the pattern I showed of different colors. That particular location, red color means very high SAR relating to the high E-field over there, of course relating to the magnetic field. So this, the

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propagation, the absorption, are all based on the local area E-field and the determine the SAR according to the sigma times E^2 divided by rho. That is the SAR definition.

So that is how it was calculated to get that capacity.

DR. WILLIAMS: What I was getting was that, if you, then, you start varying temperature and the overall temperature changes whether, still, there would be areas where you have a stronger E-field and, therefore, temperature is not going to--

DR. CHOU: The E-field does not change. That does not change with temperature. The E-field will determine the temperature but if you change the temperature, it will not change the E-field.

DR. WILLIAMS: That is what I am saying, that changing temperature through there would allow you to change on variable and not the other.

DR. FENECH: I have two questions to Ray and Graham about their whole-blood culture test that they have done. I was wondering whether one potential alternative mechanism, maybe for what you are seeing, apart from--whether it could be a pro-inflammatory effect, the whole blood, macrophages, and so on--at least with ionizing irradiation, you can get generation of clastogenic factors in the plasma, in the blood.

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So I was wondering whether you have any evidence, any idea, from scanning the literature of whether some heating might cause a pro-inflammatory response in the macrophages which might explain why, later on, you see the damage.

DR. TICE: Two kinds of answers. They are all inferential. If there is an inflammatory response, you get release of free radicals. We would have picked that up with the comet assay based on that particular assay being exquisitely sensitive, supposedly.

DR. FENECH: But the inflammatory response might kick in after.

DR. TICE: You mean after the exposure, later on?

DR. FENECH: After the comet test.

DR. TICE: In which case, we wouldn't have picked it up on that. So it is a timing effect. So I can't rule it out. But also remember that the cells that we are looking at don't produce an inflammatory response.

DR. FENECH: The macrophages--

DR. TICE: Yes; but we are looking at lymphocytes.

DR. FENECH: Oh, yes; but you can have a bystander effect.

DR. TICE: Yes.

DR. FENECH: Maybe what you are seeing is a bystander effect. Nevertheless, it is important.

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DR. TICE: That doesn't explain the 10T1/2 cells.

DR. FENECH: Not quite.

DR. TICE: Not quite.

DR. FENECH: Although you mention in your studies some effects of superoxide dismutase. You never told us--

DR. ROTI ROTI: That was in a stimulated macrophage system which was a model of an inflammatory response, as a matter of fact. But we didn't do this yet in a normal cell just trying to induce antioxidant enzymes or trying to see if we can modulate the oxidated stress response. That is one of the things I referred to in my last slide was extending that kind of an observation to a more typical situation which would be any time that enzyme is induced is it modulated, or is it just in that particular circumstance?

DR. TICE: There is an easy way to test that; that is, you use isolated lymphocytes.

DR. FENECH: There is one other measurement you could to with the cytokinesis block assay is the measurement of the nuclear plasmid bridges which gives you a measure of chromosome rearrangement which presumably are errors from breakage. I was wondering whether you measured that endpoint in the assay because that could tell you whether you had increased breakage or not without having to do a kinetochore test--in other words, if you had the slides.

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DR. TICE: We have the slides. We don't have the support, but we have the slides.

DR. FENECH: The same would apply for the embryonic cell.

DR. TICE: The 10T1/2 cell.

DR. WILLIAMS: But the slides aren't prepared for FISH or anything like that so you could look at translocations or things like that. Do you?

DR. TICE: Remember, in FISH, you could look for translocation because you are looking at DNA. We can't do the kinetochore stain because the kinetochore is destroyed the acidic acid. But you could go back and look to see if there was aneuploidy in the cell population.

DR. WILLIAMS: I have a minor point. It seems to me that a rapid way of looking at whether free radicals are involved is simply put in heavy water.

DR. TICE: Or a free-radical scavenger.

DR. WILLIAMS: Yes; I like heavy water because it is less perturbing to the biology, I think, than free-radical scavengers which can affect polyamine synthesis and things like that.

DR. LOTZ: I would like to ask a question sort of in a slightly different direction but the fact--and Ray, you mentioned this just a moment ago--the fact that, in your work at ILS, you saw changes in micronuclei with all of the

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technologies you looked at. But at Washington University, they did not.

Any thoughts about what is going on there?

DR. TICE: We actually talked about it. One of those might be the statistical tests used to drive the conclusion. What we do is a Fisher's Exact test because we are looking at the frequency of micronucleated cells which is a binomial response. You either have it or you don't.

What Joe did was he used student's t-test which takes an average of a population so his variance term had to be based on replicate cultures; is that correct?

DR. ROTI ROTI: Replicate experiments.

DR. TICE: Replicate experiments. So he looked at variability across experiments. So our particular statistical approach, which is actually normal for micronucleated data, is much more robust. So we are talking about whether or not his increase with the FDMA, was actually statistically significant if you use another test.

DR. ROTI ROTI: We will try to use that test but we were also very close to the borderline of the statistical significance in terms of--somebody asked me about the error bars and I said that the difference in the CDMA wasn't there if you just used the 95 percent confidence limits on the means. It is within those. But it was positive by the student's t-test and the FDMA one just didn't happen to be.

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But there was still a difference and the difference is close to the same absolute value.

DR. WILLIAMS: How robust is this response? In other words, if you look at other agents and how many micronuclei they can induce, are we down at the low end of those or at the high end, the middle?

DR. TICE: You can answer that a couple of different ways. First of all, you can say if I was interested in getting a robust response, what kind of facility would I be looking for.

Remember that what we saw was background in the lymphocytes, about half of a percent of cells had micronuclei. And we went to 1.5 percent. So half a percent to 1.5 percent is nothing you would--well, we got, on an average, about a four-fold increase. But sometimes I don't like fold increases because, remember Joe's control frequency was about 5.0 percent?

DR. ROTI ROTI: Depending on the cells. 2.5 to 5.0 percent.

DR. TICE: 2.5 to 5 percent. So he has got a five-fold higher background frequency than we did. So if he had to have a four-fold increase, he would be going from 2.5 to 10. We would be going from 0.5 to 1.6 and we would be calling them equally in terms of the fold increases. I am

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more interested in the absolute difference in terms of the magnitude.

If you look at those, our two magnitudes are pretty, pretty close. We are talking about a difference of 1.0 percent of the cells--not a 1.0 percent increase but 1.0 percent more of the cells have a micronucleated binucleate cell.

DR. WILLIAMS: We are still looking at fairly rare events in the population.

DR. TICE: Yes; it is just that the p-values are very highly significant because of the number of cells we are--and the magnitude of the response being low, the control frequency.

DR. FENECH: Assuming that the micronuclei originated from acentric fragments, 10 micronuclei per thousand could be induced, let's say, by an X-ray dose of somewhere between 5 and 10 centigrade.

DR. TICE: It is a really low biological response.

DR. FENECH: That is the way, I suppose, you could compare.

DR. MacGREGOR: I have another question along the same line of differences between the experiments. I wonder if those of you that have thought about it for a while have any conjecture why the exponentially growing cells were

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negative where as the plateaus were not. That seemed a little surprising.

DR. ROTI ROTI: I should answer that, I guess. I think your first question, a very good possibility is that there may have been some micronuclei expressed during the 24-hour exposure that were missed by the subsequent 22-hours cytochalasin B assay. So one way might be to go back and block during the exposure time. Or maybe expose for a shorter period of time and then block, not 24 hours, and then try that.

I don't have an explanation yet, except for one, and that is that the background micronuclei frequency seem to be higher in the exponentially growing cells so to get a statistical difference with such a small absolute change would be harder.

DR. WILLIAMS: Background micronuclei. Do they have centromeres in them, kinetochores?

DR. ROTI ROTI: I didn't measure that.

DR. WILLIAMS: But are they normally that type of aberration?

DR. TICE: Virtually all populations have both. It is just that, typically, it is about 40 percent I would say lagging chromosomes and 60 percent fragments. But it depends on the population.

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DR. FENECH: One comment I would like to make regarding the expression of micronuclei is that there is another mechanism much rarer but that can still happen under conditions of gene amplification or some rearrangements in the chromosomes.

There is an alternative mechanism that the cells can use to eliminate amplified genes. The amplified genes sort of get herded to one corner of the nucleus and then get butted out to form a micronucleus. This is work described by Shimuzu and others.

It is an alternative mechanism that occurs during s-phase so it occurs in actually dividing cells. Using a kinetochore antibody, this would look as a negative as well. It is a mechanism that we have to be aware of. It seems unlikely that it would be operating, but seeing that we know very little about what is happening, I am just making a point that we should be aware of this alternative mechanism of micronucleus expression.

DR. CHOU: I want to come back to comments before about temperature and SAR. Of course, the temperature and SAR are relating to the dielectric property. The dielectric property varies as temperature goes up and down. That was also changed. So that is a reaction still related.

For example, if you have a cup of water at 0 degrees, very cold, and you put it in the microoven, it

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will take a long time not only to reach the temperature from low to high, from 0 to 100 degrees to boil, also because the dielectric constant becomes very, very low, very lossless, not as lossy as room-temperature water. So that can be some difference.

Here, I want to mention about this 10 W versus the control that you show effects. Of course, we put the temperature sensor in there at 37 plus-or-minus, it was in that range, it seems to be, for both the control and the 10 W. That is why we want to control at 37 degrees.

But, according to the table, we have to set the circulator temperature for the 10 W to 35.3 degrees compared to the control, you need to set at 36 degrees. So the worst case, you have some cells at the very bottom of the test tubes. The worst case can have a 0.7 degree temperature difference between the two groups.

So I wonder whether this 0.7 degrees, over 24 hours, will be enough to make the difference between the two groups. I think this is all we are talking about for the temperature shift up and down whether it is sensitive enough for this 0.7 degree effects to show up in the cells.

DR. MOROS: I guess it would depend on the absolute temperature. If it is the difference between 37 and 37.7, then--

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DR. CHOU: Yes; this is the difference between the two groups. It can be worst case.

DR. MOROS: That is what I am saying. The difference between 37 and 37.7, it is different than, say, 38 from 38.7 because you have to integrate those over the 24 hours, which is a relatively long period. So the thermal dose may be drastically different. I would have to make the calculations.

But one could make the thermal dose calculation and make these evaluations, at least theoretically.

DR. HOOK: I don't have it broken down, exactly, into our 24 and three-hour experiments, but remember, from my data, that is now a 0.2 degree difference from the equilibrium temperature range that we saw. In other words, we had some experiments where the equilibrium temperature was 36.5 and we had some that were 37. So now we are only talking about 0.2 degrees difference.

DR. CHOU: That is where you measure. We try to keep that thing at 37. But, of course, there is some variation due to experimental variation. But here, when you put it into the cooling, you set that, there is a difference between the two of about 0.7 degrees according to the setting. So if the cells, after so many minutes, so many hours later, you are pretty much near the bottom of the tube.

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So, for the worst case, if they all the cells are right on the surface of the tube, the difference between the two conditions can be up to 0.7 degrees difference.

DR. HOOK: I'm sorry; what I was saying is that, in our experiments, between experiments, we have, in fact, evaluated very close to your worst case.

DR. CHOU: You cannot have the temperature sensor right on the surface at the bottom.

DR. HOOK: Why not?

DR. CHOU: When you try to measure something in a small place, always your sensor is sensing the average in volume around the tip.

DR. HOOK: The tip; right.

DR. CHOU: That is all you can measure. Usually, when you put it in, you don't go all the way to the bottom. You always do it at the bottom?

DR. HOOK: Yes; pretty well. It might come up just a little bit.

DR. CHOU: I thought you usually pulled it up a little bit.

DR. HOOK: We pull it up a little bit.

DR. CHOU: And it will be in the middle of the volume.

DR. HOOK: Right. But that is the temperature we are measuring. That is how we set it for 36, 35.3.

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DR. MOROS: I may suggest a relatively expensive way of getting rid of this uncertainty is by carefully mapping the temperature distribution with a phantom. You don't have to do the biological experiment per se, but you set up everything like you did. Then you put the probe in one place, you read it for ten minutes and then you can move it, pull it up slowly, maybe every half minute or something.

If you map the temperature through the center of the tube and then along an acentric line, and perhaps on the wall, then you will be satisfied and I think everybody else will be satisfied if you don't see that drastic temperature change. That would be a relatively inexpensive way.

DR. CHOU: This goes back to your earlier comments. When you go to a higher SAR, there is some gradient in there. So the higher the SAR, the worse the gradient.

DR. WILLIAMS: Experimentally, is it difficult to set up a system where you can maintain temperature within a tenth of a degree or two-tenths of a degree? If you are foreseeing experiments where the variable is exact temperature, how precise can an experience system be set up to maintain that over 24 hours?

DR. MOROS: In the RTL system, as you saw, the RTLs were almost 4 feet by 4 feet in size. There are

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16 flasks and there are 9 RTLs. So there is a challenge in terms of temperature control.

I can confidently say that, at a steady state, once a system is running for a while, there is a variation of 37 plus-or-minus 0.3, 0.3 not being the standard variation, being the maximum deviation.

The reason for this level of control is because of the aluminum plates. They are large thermal-conductivity plates that homogenize the temperature over a very large area. Now, the situation with their experiments and with other experiments is that once you have isolated mediums floating in air, then the way you cool it is somehow there is some air convection around the tubes or around the flask. Then temperature control becomes more of a problem.

But I believe what they said is that they control it at least at that point. That is why I suggested that if he maps the temperature and convinces everybody that within his biological sample, you can map that relatively fast, that may be even in a day, it is enough data to convince everybody.

But the gradient that will be sustained within a test tube in air, or in a flask in air, are much greater than the gradient that will be sustained against an aluminum plate, no doubt about it.

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DR. TICE: If you are not talking about doing an RF exposure at the same time, but you are just talking about maintaining temperature, then the setup that was used, in the absence of an RF exposure and where the room fluctuations were controlled.

Like, you put the TEM inside an incubator. Or you put it inside a water bath, itself. The way we are doing it is, if we kept control under those conditions within 0.3 centigrade, we should be able to go less than that because we can get rid of what is causing that variability. So you should be able to get down to a tenth, or two tenths. I think, practically, it is possible.

DR. WILLIAMS: You might want to consider, although they are expensive, the infrared cameras between 3.0 and 5.0 micron sensitivity. Then can look at the whole flask and give an instantaneous reading of temperature within about a tenth of a degree and they can take any number of images with time.

It might be worth trying to do, just a detailed study with background temperature being varied at different SARs, simply the patterns of temperature on the surface of a plate as a function of the time of exposure.

DR. MOROS: You want to be careful when you use an infrared camera because what you are looking at is surface temperature. In the case of a test tube, then you are

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looking at the non-flat surface so you are looking at the optical--

DR. WILLIAMS: If you move up to 3.0 to 5.0 microns, you are looking at the black-body irradiation and you really are looking at the average temperature within surface. If you have a fairly low surface, that is not a problem. We look at tumors underneath skin and can see the temperature in the tumor very well.

So I would agree that, if you are working in the near infrared where things are transparent, I would agree. You get reflectants. You would get all sorts of surface phenomenon. But if you look at the longer wavelengths that are measuring black-body emissions, then I think you can get a pretty good indication of the temperature of the substance, itself, and not a surface phenomenon.

DR. CHOU: Also, you are talking about the difficult thing here is doing irradiation, everything is within an enclosed chamber. You use infrared to try to look--that is hard to do unless you open everything up.

DR. WILLIAMS: I don't know if you can, but all you need is a hole about that big for the camera lens. I don't know if that is possible or not. I saw windows and things.

DR. CHOU: That is for the air to blow through, but to try to get something in there is not easy to do.

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DR. MOROS: I really think that that type of infrared camera--he hasn't told you the setback of trying to get that type of infrared camera. We are talking $100,000, which is probably a year of anybody's grants.

I really think that an inexpensive way to do this is to set up your experiment at 5.0 and 10.0 kg, and sham, and map the temperatures up and down every--I have done this in tumors. I have done it in kidneys. It is not extremely difficult to do and that really will give you the data to convince yourself and everybody else.

DR. WILLIAMS: Sometimes the cheapest thing is to get the right answer. So I could see that maybe FDA would have one of these cameras that could be moved to different sites. At some point, you are really going to want to do interlaboratory comparisons where you use the same measuring techniques to see the patterns of--it would seem to me that would be a logical line of procedure that would come out of this.

But you are right. A good camera is about $50,000.

DR. ROTI ROTI: We still have that camera; right?

DR. MOROS: Yes.

DR. ROTI ROTI: It could be done with their system as well. What Eduardo used for the in vivo studies was a splittable phantom where, right at the end of the exposure,

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you pull it out of the exposure chamber, split it in half and then image it with a thermographic camera.

To image the flasks, they had gelled media with an open top, and you immediately lift the lid up and image the flasks. So they could take those test tubes with splittable phantoms in them, pull those out and image them with a thermographic camera.

DR. CHOU: Joe, for that kind of an infrared--for that kind of technique, that is usually for dosimetry purposes. We have done this for many years. That is usually for a very high-power level in a very short time. You split the model and you measure the measurable temperature rise.

We are talking about a little tiny power now. That is very difficult to do. By the time you open it up, all the heat is all gone.

DR. WILLIAMS: You don't think it is possible to monitor it real time through a design apparatus so that there is an aperture for the lens underneath the flask, and just for the studies of following temperature at different SARs and different times would be possible?

DR. CHOU: This is inside the chamber, and it goes through all the flasks, all the plastics and all the reflectants. It is not easy.

DR. HOOK: It is very difficult.

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DR. MOROS: The only upgrade that I would suggest is that instead of using a thermistor, you would spend the money on a Luxtron system.

DR. HOOK: We had a Luxtron.

DR. MOROS: So if you have that already, I would just take the time and do a map under the exact conditions of the experiments, and even including 24 hours. You can map and then go back to the first point and then map again, and then go back to the first point and map again.

I would be convinced of that kind of data if you chose that the temperature within, during your what we call the "value of interest" is within 37 plus-or-minus--

DR. CHOU: That is doable.

MR. BASSEN: This is what is routinely done in hyperthermia. Luxtron probes were developed for that purpose. An array of 10 or so probes can map points in a small temperature elevation so you can get real-time data.

DR. HOOK: This is something Ray said, and I think we could do this, is instead of doing multiple assessments, put a whole bunch of probes in the tube. In our system, we had four. I think you had a 16-probe system. You could put all 16 probes in the same tube.

DR. CHOU: If you put too many, you are going to replace the liquid by the fiberoptic--

DR. HOOK: I don't know. How spots do we need?

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DR. CHOU: You don't want to disturb too much of the medium and be enough to get multiple points.

DR. HOOK: The other way is we are looking at either opening it and moving it or doing another experiment every single 24-hours and moving it one stage at a time.

DR. MOROS: No, no, no. I didn't suggest that at all. You start your irradiation with a probe inside. You already have some sort of micrometer system attached to the probe so that you can move the probe up.

DR. HOOK: Not without opening the whole--

DR. MOROS: You can drill a hole in this thing.

DR. CHOU: Yes; we can do that. We have the holes on the door.

DR. TICE: The holes are there.

DR. CHOU: You can pull it, by pulling a millimeter or so.

DR. MOROS: And then what you do is you take the time every twenty minutes, every half an hour, every hour, you do a map. You go up and down. You record the temperatures. And then you wait another half an hour. It is still irradiating and then you do it again, for 24 hours. In fact, you may not need to go 24 hours, but you need to map the space.

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DR. CHOU: I think half an hour or so, it would be all equilibrium. You would just have a thermal gradient in that area.

DR. HOOK: Maybe if we can go backwards a bit because we seem to be getting maybe too specific on what we can do to evaluate our system. But I would, again, maybe to get things started again, one set of our data, at least for one technology, our fold increases were similar. We found the same effect for 10 W/kg and 5 W/kg.

DR. CHOU: That is expected. Due to temperature, it should be the same, because your SAR is the same.

DR. HOOK: 10 W/kg and 5 W/kg.

DR. CHOU: Yes; in CDMA, TDMA. It doesn't make any difference.

DR. HOOK: No, no. I am saying, for one of our technologies, at 10 W/kg, we induced the same level of increased micronuclei as we saw for 5 W/kg, exactly the same. Surely, the number of cells that could possibly be at a high SAR should be different between those two.

If that is the case, why are we seeing that? If it is related to temperature, why aren't we seeing a decrease?

DR. CHOU: That is only one experiment.

DR. MacGREGOR: I wonder if I could introduce a slightly different question. I think it important to

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understand what is going on in the current experiments which is mainly what we have been discussing. But I think another key question, maybe the important key question, is can the observation that has been made be replicated in vivo. I wonder if there are existing exposure equipment that would enable in vivo experiments do be done under the same irradiation conditions.

DR. ROTI ROTI: The answer is yes. I mentioned that we did a 9-L tumor-growth study for an implanted brain tumor. We have just completed the two-year bioassay, exposing the rats for 4 hours a day, 5 days a week for two years, and the histology is just being worked up.

We still have all those exposure chambers and we could use that system to expose rats to these signals and see, with either the bone marrow or the blood assay, if micronuclei were induced. So the answer is we have the exposure systems in place to do in vivo studies.

DR. HOOK: What is your maximum SAR, though?

DR. ELDER: That is a point the needs to be made. You probably have the systems to do the in vitro experiments, but you can't expose those animals to 10 W/kg for 24 hours, or you are going to kill them.

DR. ROTI ROTI: We have some guy-type irradiators, but those are more for 2450. So it is hard to do the high

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SAR. You would just see if a more chronic experiment at low SAR induced it.

DR. LOTZ: There is some, I think, significant value in looking at a lower SAR a longer time in vivo from the sense of--I was pondering earlier the question, when Luc presented his talk this morning, he referred to a couple of occupational studies. Those clearly are not getting 10 W/kg or anything close to that. In fact, we don't really know because SAR is not an assessment in those. They are presumed, by virtue of their job, to have been exposed.

But if there is any validity to those findings, there is something going on in those individuals. There may not be validity, but that is certainly an open question to be pursued.

DR. ROTI ROTI: The sad part is that we had terminated the 2-year bioassay when these issues came up. Otherwise, we could have run the bloods on all of those animals.

DR. TICE: Joe, you don't have slides for hematology?

DR. ROTI ROTI: I don't know if--I would have to go back and check. I can ask Marie.

DR. OWEN: It seems like doing those in vivo exposures would require a lot of front work in terms of more

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thoroughly looking at the dose response and the time course from the in vitro experiments.

DR. ROTI ROTI: I guess it could be a second-line study, I suppose. But, just like killing the rats before we found out about this, there is pressure on us to disassemble that facility.

DR. WILLIAMS: Certainly, I think the 2-year bioassay is still the standard, first-line for predicting cancer. But I do think we have the problem that we were talking about with cells, if you have a athermal and thermal effect, you can't set the exposure limits in a bioassay that you would normally by taking a 7-day toxicity and dividing.

So I think it is important, before we can interpret a bioassay, to know something about uncoupling the thermal and athermal effects because I am sure a precedent to the study would be sort of detailed dosimetric studies in the intact animal, seeing what kind of SARs you are going to get through the different tissues.

There something that I'm sure you consider is the problem of scale. Making little bitty cellular phones is what is expensive for the rat experiment.

DR. MOROS: Our system used a standard microwave antenna and the rats were positioned angularly around this antenna with the nose of the rats approximately 3.5 cm from

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the center of the antenna. The dosimetry paper was published, I believe, in either '98 or '99.

From that paper, you can see the SAR distribution that we were able to attain. It would be just a matter of cranking up or down the power. The power level we use, I believe, was 1.25 W to each antenna. I don't recall the average SAR value. But it is in the literature.

So we have, what, twelve of these chambers, four for each one of the signals and sham. Each one of them can hold up to 40 animals. So we were running a 440-animal experiment. Obviously, for another type of study, we probably don't need that many animals.

So we could even share. I don't know. They are still there. I don't know how long they are going to be there.

DR. TICE: Russell, do you happen to know--isn't NTP, right now, trying to schedule or review or come up with a conclusion whether or not they are doing an RF exposure for their bioassay?

DR. OWEN: Their executive committee approved our nomination for study and it is going into the next phase of their process which is a two-stage, as I understand it, finer look at the exact study design, feasibility and even costing.

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DR. TICE: So then one of the things that might be useful in the long run is to schedule some, let's call it genetic toxicology endpoints as part of that assay, which they tend to do anyway but they don't do all the time. So that might be a useful thing to try and get some of this additional data from.

DR. WILLIAMS: Has there been any discussion yet on how they are going to set the dose exposure levels or exposure patterns?

DR. OWEN: No.

DR. FENECH: One point that was raised earlier in the talk by Luc Verschaeve were the synergism-type experiments which were done with mitomycin C and X-rays, I think. In the human situation, at least, and also in the mouse, another factor that can induce micronuclei is folate state, or folate level in the culture medium.

Now, this is of relevance to the human exposure situation because folate status in people varies, and there is are also genetic differences in key enzymes that metabolize folic acid, not to mention the DNA repair ones.

One possibility for the interaction could be, let's say, if heating is occurring, to some extent, or could cause depurination of the DNA, let's say, or causing increased excision-repair activity that could synergize with the folate uracil incorporation is a possibility. I was

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wondering if we shouldn't keep this in mind seeing that this is an important variable in the human population and also the fact that there are, for example, other DNA-repair enzyme defects, let's say the RCA-1 mutation which is relevant to breast cancer is also associated with increased micronuclei induction, let's say after exposure to ionizing irradiation.

So these are the aspects. After all, it is very likely that those who get cancer are probably predisposed in some way or another, anyway, as it is. The selection of the types of cells we use in the tests can--considering, for example, that Ray Tice was one of the subjects in one of those experiments, you could argue that Ray Tice is not necessarily representative of the sensitivities of the general population.

DR. TICE: Absolutely.

DR. FENECH: I think there is a point here that if any tests are going to be done with human cells, then the nutrient status of the culture medium and possibly the DNA-repair capacity of the chosen volunteer, and so on--or at least to have volunteers with different capacities or repair.

The obvious example would be a BRCA mutant as opposed to one that isn't.

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DR. WILLIAMS: We have just surveyed the literature to identify populations, syndromes, with increased cancer risk. We found a little over 240 of them. For those that you know the gene, and have sequenced it and its function, about 75 percent of those are deficits in control of proliferation. Only 25 percent of them have to do with DNA fidelity, which might expose--so the question of whether you test for the sensitive population or the general population is one that has been discussed a lot.

If we knew the mechanism, then we might be able to confirm it by selecting individuals that are know to have defects in certain repair pathways. I can't even find out the genetic background of people I study. I am not permitted to. I just have to get them double blind from the laboratory.

You can get, say, people with BRCA-1 or BRCA-2, but you really have to go through long genetic counseling and permission. I think, perhaps, from our studies--we have only done 17. You have done many more donors than that by now, I am sure. And we always do a fluctuation.

So there is a high background difference, particularly in gaps and breaks, less in acentric fragments and dicentrics in rings. But, generally, the background is quite low in acentric fragments in all the people we have looked at.

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So I don't know if that is a problem at our level of testing. The variation we see is not so great that I think it would confuse the results at this stage.

DR. VERSCHAEVE: I think if you look at the data for the synergism--one of the studies, anyway, the one that we performed, was on ten individuals and it was very reproducible. So, of course, I don't know if ten individuals is enough to have differences in genetic variability or so. But, anyway, what I want to say is that in that study of ten individuals, we had the same response.

In another study, from, again, I don't know exactly how much, but several individuals, we have, again, the same response.

DR. FENECH: So each individual showed an increase.

DR. VERSCHAEVE: Each individual showed an increase and one individual maybe a little bit more. But the overall result is the same. But maybe ten is not enough.

DR. ALLEN: I would like to mention one other factor, too, that could pertain to the choice of human lymphocytes would be age, because it would be pertinent with regard to HSP70, with its response to heat. It has recently been shown that it declines with age, the HSP70 response, to heat and other agents.

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So it could conceivably be a factor with the heat induction here.

DR. HOOK: I think repeating it somewhere else is going to be a big issue. Certainly, bringing in other donors would help. We did have two donors. I don't want to say the other one was significantly younger than Ray, but he was younger.

DR. TICE: Russ, has FDA talked about tying into the NTP center for microarray technology and trying to see whether or not they can see what genes are upregulated or downregulated in a population of cells exposed.

There are a lot of problems with it just in terms of interpretation right now, but if you sort of get it on board in that direction at an early stage, at least you might be able to find whether some of the patterns where you look at multiple genes at one time might be informative in seeing, like, for instance, if there is oxidative damage or if there are heat-shock proteins and all the other things being tied--

DR. OWEN: Yes; there are a number of studies that are under discussion associated with that nomination to the NTP and, while we haven't, of course, set anything up because it is still in the early stages of consideration, that is a good idea to consider and something that we have thought about.

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DR. LAGROYE: I have a question. We are speaking right now about SARs that are at least twice the SAR people experience using the mobile phones. My question is, can we expect that metabolic exposition to low SAR radiofrequency can lead to the same effect of high SAR radiofrequency.

DR. OWEN: Do you have, from your data, any information that bears on the question of whether multiple exposures at a lower SAR equate in any way to higher exposures?

DR. TICE: No. Basically, the positive response was the 5 and 10, where it was a 24-hour continuous exposure, and the one didn't do anything. We didn't do 2.5 which was our other SAR that we had tested in the short, the 3-hour exposure, setting.

But one of the questions that, of course, that you worry about when you talk about extrapolation to people is whether or not we are looking at an event that can accumulate across time as you get multiple exposures.

The only thing that bears on it is the data Luc talked about from the human studies and I guess the cow studies where they saw an increase in micronucleated erythrocytes in cows and an increase in micronucleated lymphocytes in the people. I have never seen those studies, in the sense of actually trying to evaluate their scientific credibility, but if those things, in a sense, those "stand

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the test of time" or are credible, then those probably have the biggest bearing in terms of whether or not an increase in micronuclei is a biological event which is relative to human exposure.

DR. FENECH: Those particular studies would have to be replicated because there has been control for dietary factors that impact on the micronucleus index.

DR. TICE: Especially in the cows.

DR. FENECH: Well, the cows probably are eating the same thing. If anything, that is probably the most robust, I would think.

DR. VERSCHAEVE: They are eating the same thing, but maybe they are in a polluted area, and that I don't know.

DR. FENECH: Going back to the in vitro lymphocyte assay with the primary lymphocytes, it is possible to culture them for up to 9 days, maybe 14 days, so you could do a chronic lower-dose experiment, I suppose. Of course, with cell lines is another possibility.

DR. HOOK: We could do split dose, or anything you want.

DR. MacGREGOR: Just to get back to one of my earlier comments, I would be interested to see an experiment where the exposure continued all the way through the cell cycle and through our seeing an effect and you don't really

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understand how to maximize that effect at this point. It would be good to understand.

DR. WILLIAMS: Comparison of the lymphocytes to the cultured cells gives you some indication there where the lymphocytes are irradiated in a non-proliferating stage and the cultures cells are proliferating. 24 hours is about 80 percent of the cell cycle.

DR. ROTI ROTI: It is more than a cell cycle for 10T1/2 cells.

DR. WILLIAMS: Oh; that's right. They grow rapidly.

DR. ROTI ROTI: So even 16 hours was a cell cycle. Maybe what we should have done was added the cytochalasin B after the quickest cell cycle so that the fastest cells would have been through one cell cycle and the slow ones would have been somewhere behind that. And then we could have pulled them out for an additional time to get up to the 22 hours.

So that is an interesting protocol for the exponentially growing cells would have been to, maybe after 16 hours, add the cytochalasin B.

DR. TICE: Actually, Joe, what I would do is add the cytochalasin B at the same time you start the exposure and do two sample types. You are going to get both early

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binucleates and late binucleates coming out that way and you still only look at binucleates.

DR. WILLIAMS: We can do two cultures, one with the cytochalasin B from the time--and one after.

DR. ELDER: There has been a lot of discussion this afternoon about some fairly exotic experiment work. I guess, if you put a price tag on what I have heard, just the animal work and the in vitro work would be in excess of $10 million. That doesn't include the human stuff.

I think some of the experiments are interesting and should be talked about, but I think we need to remember that at the present time, we need to find out if the experiment we are talking about here can be independently replicated. That is more or less step 1 in the process, I think.

We need to spend a little time talking about maybe what is the best way to go about doing that.

DR. ROTI ROTI: I have a short proposal, although I am not a PI. But, actually, a lot of the expensive engineering has already been done. It may not need to be replicated if we want different groups to replicate experiments already performed. So we could work out some sort of agreement where we swap the engineering system, the irraditor systems, or swap the investigators going to a different place. Either way. That would save a lot of

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money and at least different people would be able to duplicate the experiments.

I just wanted to reemphasize, duplicating the reeingineering system is a big portion of the money.

DR. CHOU: We are not lacking of the chronic studies. Quite a few studies have been done in the past, starting from the Air Force time on the effects of the radar RF exposures at the University of Washington, Georgia Tech and Frye from San Antonio. On the cellular phone, quite a few are under way now. So there are quite a few just kind of long-term studies have been going on or have been already completed a long time ago.

DR. LOTZ: I guess one of the things on there would be whether or not any of those bioassay studies that are under way could even now back into maybe doing something with micronuclei like Joe was just saying he wished he had done now before he killed the animals and dismantled the system, or whatever.

So that might be one possibility. I don't think--the literature that exists on past studies certainly doesn't help us much on this particular point, anyway. One of the limitations of some of those other studies, although they were well done, was that they were a single dose so we don't get any dose response information.

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DR. TICE: Does NIOSH have an exposure facility for this kind of wave length?

DR. LOTZ: We have a brand-new RTL system. We don't have anything for in vivo.

DR. TICE: No; I understand that. But at least if you have got the exposure set up for the in vitro, because the in vitro is what we are trying to replicate.

DR. LOTZ: That's right. In fact, we are in the process--as of last week, we have all the pieces, literally. We just got our amplifier.

DR. OWEN: A related question. The exposure system that you used at ILS, is it still there? Is it still intact?

MR. WILLIAMS: Can I answer that, Russell?

DR. OWEN: Okay.

MR. WILLIAMS: My name is Dylan Williams, for all of those who know each other and don't know me, because I am a newcomer here. I am just a business person. I have purchased a WTR exposure system. I purchased it for the betterment of what I am hoping that the scientific community can move forward with it. I didn't want to just see it fall into a box and disappear.

I have been following this on the sidelines pretty closely, only through the literature. I really haven't made any contacts, but I am hoping that, through today, through

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some discussion, we will be able to have some concurrent discussions going forward to make sure that that exposure system is also available and in use because I think it was a very valid exposure system that was put together with a considerable amount of money and could be very useful.

I also want to discuss it further so that the correct people are using it; in other words, the people that know how to make it work, the people that have done the bioassay work, that understand. That is why I am here today. I just don't want to go out there on my own and start saying, "Hey; we can do these kinds of experiments for you guys." I want to work with you guys.

So, to answer your question, it is actually still sitting in a box at Ray's house, so to speak.

DR. OWEN: Do you want to buy some chamberettes?

MR. WILLIAMS: Yes; I might want to.

DR. WILLIAMS: The recent sort of painful experiment of the rapid program--I don't know if it is a painful experience or not; a lot of people did a lot of scientific research, a lot of negative results. That is sort of the top end of replication where you hire people independently to reproduce data.

There was a real--I think it is different from what it is here. In the early stages of the rapid program, I think there was some, at least in the back of some

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people's minds, the idea that some of the results were not done correctly. I don't think that that is the case here, at least from my own judgment.

So there is the repeatability problem. You need to repeat it more than two times. That is clear. And you can repeat parts of it, although it seems to me that the critical part would be the exposure. You could send slides to people to read micronuclei but that is not something that differs very greatly between laboratories.

You could use the same donors. You could split cells. You could use the same stocks. You could use the same media and everything. It seems to me, just from an outsider's viewpoint, that it is exposure systems that vary predominantly between the different--and I am just not qualified to speak--I'm sure Dr. Chou could--about how different this would be and whether you would expect different distributions of temperatures and different distributions of SAR. And we have seen some indication of that.

One thing that bothered me very much in the rapid program is they went outside the people who were interested in this area, who were very good biologists, to reproduce this and they spent huge amounts of money on different exposure systems with the idea that a common engineering group had to go visit that place and certify that the

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exposure apparatus was producing the changes in fields that they wanted to measure.

I don't know where we are compared to that or where FDA wants to go compared to that. But repeatability and reproducibility can be a huge project. So I think what we have to do is proceed in a very careful, thoughtful way. and what are exactly the critical questions in approaching them in a sequential way.

My personal feeling is we are pretty far from in vivo studies. We have to know more about thermal versus athermal mechanisms so that you can interpret the patterns in the animals and know what tissues you are really looking at in terms of possible biological effects.

DR. HOOK: I guess maybe because I am a biologist I would argue the opposite. I think we have got two exposure systems and we have seen somewhat--at least these results are not inconsistent with each other and what we want to evaluate is whether the biological response we are seeing is robust.

So we want to repeat it with other donors in another lab using different culture media, different sources of serum, different everything. If it is a robust biological response, it will show up in those circumstances. If it is not, then it is something that is particular to our

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laboratory and our culture conditions, and then it has less significance.

So, to me, if we have got one exposure system and shift it around to different people, and use variations on their biology, that tells me it is a much more robust response than worrying about the exposure system that we have had.

DR. ROTI ROTI: I think if we can get comparable SARs with the two exposure systems, it would go a long ways to answering this question. We have a little bit of a difference. I think we have a little bit clearer temperature, a more homogenous temperature situation, but a limit with the current design for the SAR, we can achieve.

The TEM cell with the test tubes in it has questions about temperature homogeneity but can really go up with the SAR without too much problem. So what I think we need to do, at least from the RTL--I don't know how much a project like that would cost. Actually, Eduardo knows how much that would cost--but do try to improve the SAR output of the RTL system so that we can maintain the temperature control that you have seen so far and yet do a comparable SAR to what Ray and Graham have done.

DR. WILLIAMS: Are the investigators convinced that, by varying temperature and pressing with RF that you will be able to discern a thermal from an athermal effect?

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DR. HOOK: To speak first, I am not convinced. To start with, if you drop the temperature too much, there is evidence that hypothermia induces micronuclei too, so you might have a problem. We don't have a lot of data either way.

DR. ROTI ROTI: I think the nonthermal and thermal effects can be distinguished. I think we have lots of strategies that we have discussed already to get at that either by temperature, modeling temperature alone studies, heating with the microwaves, heating without microwaves, and then trying to actually monitor the temperature with a biological probe.

I think all of those are ways of getting at that. So I think that is a doable part of it. I think that trading systems, and things like that, with the different exposure systems is also an approach that we can consider.

DR. FENECH: I still have a problem with the idea of separating the thermal and nonthermal effects. If a particular type of irradiation is producing a thermal effect as well as a nonthermal one, then it is a bit like a complex mixture-of-chemicals, so to speak, effect.

So you have got two events that are happening. I mean, in vivo, if you are exposed to this particular radiofrequency, it might produce both effects simultaneously.

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DR. ELDER: I think we have to do that, too.

DR. FENECH: So I can't understand, if you are trying to evaluate risk, why you want to separate the two. if that is what happens in vivo.

DR. WILLIAMS: To use your complex chemical approach, what we try to do is take each chemical separately and see the effects and their combined effects. When we go to an animal, at least my impression is that we will have a wider variation in SARs through different tissues.

We can't get a homogenous dose through the whole animal as we try, so it just seems to me that, down the line, you are either going to have to take a purely empirical approach and say we are going to take a lot of biological systems and we are going to put them under the rays and see what happens.

Generally, that has not been a particularly good mechanism in toxicology, at least from my viewpoint. You get the basic toxicity studies but, before you go to the higher end, which can be $10 million. For instance, if you do fractionation, multiple exposures every day, for how long, all of those problems are very difficult to assay or to approach or to even set up good toxicological experiments unless you have some idea of the mechanism.

In other words, if there is a thermal and athermal effect and you want to know whether certain exposure

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patterns produce thermal effects in the brain compared to the spinal cord compared to the ear, it just seems to me that, without being able to separate those two, the way is very long and hard and difficult to interpret.

If you are saying that, then you are simply going to drive it empirically and say, we will use this as a single agent, as a mixture. Then you are going to drive yourself very quickly to the animal experiments, and there will be a fairly large number of them, and then to epidemiology.

It seems to me this meeting is important in deciding the directions we go to reach those decisions. So I really strongly feel that we have to try to understand the mechanisms as best we can.

DR. MOROS: I have a question, probably, for the FDA. Dr. Roti Roti thinks that we can separate the thermal from the athermal SARs. I agree with him, partly. I agree with him up to a certain SAR. There will be an SAR for which we will not be able to control the temperature in a fashion that we will be able to extract the heat generated in the system at the same rate that the SAR is inputting and entering into a system.

Therefore, we are going to have temperature increase or we are going to have gradients within the sample, which means we have temperature nonuniformity. So

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there will be an SAR at a practical level where we won't be able to separate these two.

So my question was what would be the upper bound SAR that is of interest to the FDA. For cell phones, it is probably 2 W, 3 W. Why do you want to go to, say, 10 or 15 W?

DR. OWEN: The idea, I think, here, is not to try and model the exposure that is being encountered in the environment but, rather, to determine the existence, the robustness, the mechanism of a biological effect. To do that may require going far outside what you would see in environmental exposures.

In a, perhaps, poorly analogous way, you don't do toxicology studies at environmental doses of chemicals. Generally, you go up to fairly high levels.

DR. WILLIAMS: Something I mentioned earlier. It is the dose-response curve. Certainly, one approach would be to go down to levels of exposure that you predict would occur in human beings. But we cannot expose the number of people who are going to be exposed.

We have to get a dose response so we can expose fewer animals, 100 animals, at most 1000, and extrapolate that effect down to low doses. I think many people here would guess that if we would expect to see nothing in the number of animals that we could appraise for carcinogenesis.

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So the whole idea of getting a dose-response curve is so that you can get a dose response and extrapolate it back to proposed exposure levels.

DR. MOROS: I agree. My point is that when it comes to radiofrequency and the thermal and athermal, there will be a practical limitation for the upper-bound SAR.

DR. WILLIAMS: That's right. The overlap, if there are two effects.

DR. MOROS: We will not be able to separate them.

DR. WILLIAMS: But if we know that there are two effects.

DR. OWEN: That is also part of the reason for considering experiments that are non-RF, heating experiments.

DR. MOROS: What I mean is that there will be an SAR value, an exposure value, at which you won't be able to control the temperature at 37 degrees. That is what I am saying.

DR. WILLIAMS: I agree. But that is going to be above the predicted human exposure level for a single continuous exposure. But, again, if you are going to try to interpret the question over here, multiple exposures over a long period of time, then how do you transmit protracted or fractionated exposure to continuous.

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You only do that if you are sure that the small exposures are working through the same mechanism as the continuous and the high-dose exposures.

DR. ROTI ROTI: I think the only thing you can do when you start having these high thermal gradients and those questions are present is to determine, at a given temperature, what the measurable effect for endpoint in question is and then ask, when you are creating that same temperature rise with an RF field, is there any difference in the response. Then, that increment would be the nonthermal component.

DR. LOTZ: In fact, to follow up on that, Eduardo, it seems to me it would be relatively straightforward to do the temperature study in the RTL, just raise the whole temperature in there in the system and see--if you did the RF study that it was high enough that you couldn't hold the temperature down, but it only went up maybe a certain amount and then you did the thermal non-RF study, raised it the same amount but got a lower effect, you could say, "Well, the RF has a greater effect for the equivalent temperature increase," or something like that.

That kind of comparison seems possible to me.

DR. MOROS: I agree. Then I will go back to, I think, perhaps, my very first comment which was we need to chart the waters, we need to chart what happens in terms of

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micronucleus expression. In terms of temperature, we don't know what happened. If we had that data today, we would be discussing it, because it would be very relevant. But we don't have it.

DR. WILLIAMS: Would you look at an interaction curve for standard agents that produce the different types of micronuclei; for instance, heat with a spindle poise and heat with ionizing irradiation to see--

DR. ROTI ROTI: No; we haven't done it in the lab, but we could. One of the things we have ongoing, because we didn't really start out as a micronuclei project, but we have considered other things. One of the famous putative RF effects have been perturbations of cell proliferation.

We basically have begun a study--actually, it has been ongoing; it is almost finished--of single temperature rises, single-degree temperature rises, on cell proliferation. We have all of that technology in place because it was not straightforward.

Basically, we needed a humidified transfer box so the temperature didn't drop when cells were being transferred, and there is a whole range of that technology. So a fairly well-controlled temperature-only perturbation system is in place that we have already built to look at those kinds of thermal artifacts and characterize them.

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So I think that, looking at very small temperature changes in terms of the generation of micronuclei is probably an important baseline because, as we found out from cell-proliferation studies, surprising differences occurred. In other words, the first temperature rise does not inhibit cell-cycle progression. It stimulates it.

So if somebody found an inhibition of cell-cycle progression with a microwave field and somebody said, "Oh; that is a heating effect," well, no; it should have stimulated it before it inhibited it.

DR. WILLIAMS: Sort of a theory of RF harmesis?

DR. ROTI ROTI: Right.

DR. CHOU: Of course, temperature-control experiments are vitally important. I want to emphasize again, we are not shooting for the final temperature the same. We should also try to mimic the rate of temperature rise because the two can be very different.

DR. OWEN: Although, in the absence of much data on the response of the endpoint to non-RF heating of any sort, if it becomes prohibitively difficult to model RF heating by a non-RF method, one may have to cope with that limitation and still do the experiments to get the data on thermal effects, non-RF thermal effects, non-RF thermal effects.

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DR. CHOU: In the early '70's, I was playing with all the different nervous tissue or muscle tissue and I couldn't see anything different. If you can really mimic the temperature rise and all that--depending on the system you use, you may have trouble trying to simulate the heating. But the system I used was very easy, just produce the temperature and they are very close.

DR. HOOK: Those are circumstances where you are looking at just one nerve or just one piece of tissue.

DR. CHOU: Yes.

DR. HOOK: We have a population of cells in the bottom of the test tube all of which have different temperatures. Presumably, that is the theory. I don't know how you mimic that by warming up the entire tube.

DR. WILLIAMS: Hopefully, you will have cells that represent each temperature variation and see whether there is a dose response for temperature to induce micronuclei and then compare them to the fraction of cells in your exposures that have that temperature or above. I think that would be the way--

DR. CHOU: Originally, we designed the system, TEM cells for the Petri dish. The bottom of that circular ring is very homogeneous. But, because of the procedural restrictions or limitations, the lab has to use the other tube. That makes it very complicated.

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DR. MOROS: That is why I make my biologist work the way I wanted her to work. I told her what to use. She had to use the T-75 flask with 40 ml of medium.

DR. ROTI ROTI: We chose the T-75 flask because it is the closest to the 100 ml Petri dish that is used in the standard neoplastic transformation.

DR. OWEN: I would like to invite additional comment on something that, really, Dr. Moros raised in a question earlier. There have been two or three comments suggesting that, to go further, one thing that is really essential is something that people have been talking about for a long time so, perhaps, we haven't talked about it as much this afternoon, but that is the closer characterization of the dose response of the bioeffects at hand.

So I, again, would like to invite additional comment on what range of SARs might be most useful to test in order to get a better handle on this, how close the steps should be, and any other ideas.

DR. HOOK: We haven't really, from our data, produced enough that you could see there is a dose response. we have got two experiments at 5 and 10. In one, they were equal and one we had a little bit of a dose response. So that is what we have.

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So we would either have to go somewhere between 5 and 10 to see if we could produce something, repeat, do more 5's, or go higher than 10. Go 1 to 10? I don't know.

DR. CHOU: For practical purposes, this is different from a toxicology approach. You try to go over a very wide range and try to find out how toxic anything can be and then divide it by a million and things like that.

So, for the SCC28 Subcommittee 4, we have tried to come up with a safety standard for human exposure. So far, the whole body is 0.4 for humans and 1.6 W/kg with 1 G PK SAR for uncontrolled conditions and 8 W/kg for controlled conditions. That is the current limit now, and also the ICNIRP is using the same numbers, 10 and 2 W/kg. So it is all in that range.

If any study goes up higher than that, it doesn't really mean too much for the safety standard because we already know above that is not allowed. We don't want to be higher.

DR. LOTZ: C.K., I would jump in here because, to me, that is kind of--you have got it backwards. Yes; we do know that 10 is way beyond what we want to allow through whole-animal work. We would not want to allow whole-animals to get 10. But I still think we need--I can't say it much better than Jerry did a few minutes ago about why we need to push that dose response to see, to understand the effect.

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I don't think we actually have enough strength to our biological database to the 4, and particular to the 1.6, to let them be the driving factors in this. I think we need to make the biological endpoints we are concerned with be the driving factors in what we study, not where we are in numbers in existing documents.

DR. CHOU: I am saying these as reference numbers. When we designed the study, we use 10 W/kg. We try to be high to cover some range. But another question is should we go even higher than 1 W/kg.

DR. LOTZ: As Ray mentioned, I think 1 to 10--I think there is a lot of merit in that.

DR. CHOU: Yes.

DR. LOTZ: Clearly, both labs indicate something may begin to happen about 5. I almost think we have got to go above 5 to see whether that is real, whether it holds up.

DR. CHOU: We designed both studies for in vitro, and also the in vivo for the DNA study. They both had 10 W/kg as a maximum. I think that is reasonable.

DR. LOTZ: I do, too.

DR. TICE: I think what we are trying to arrive at for a dose-response curve is the shape of the dose-response curve, in the sense of is there a plateau or not a plateau, because that might be in formative. The second thing is where the breakpoint is.

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The other thing to reflect on is that, in both cases where we tested one, there was an increase. It just wasn't statistical. Statistical is driven by the number of cells we score. So one question is, just like if you do more animals for cancer research, do you get a different threshold or a different break point.

So what I would be looking for is a dose response between 1 and 10. Then, as you start accumulating data, you start going down to either lower doses or you start collecting more data.

One way of doing it is, rather than scoring the number of cells to arrive at the micronucleus is to score a set number of micronuclei and then figure out how many cells that takes. Then what you do is you end up with actually more robust data as you go down to lower doses; just different approaches. But I wouldn't be stuck on scoring 2000 cells. That is just because that is the standard assay, but it is not necessarily the most sensitive way of handling the data.

DR. HOOK: The only reason I brought up going above 10 is that, although I am interested in the break point, I am also interested in seeing if we have hit some plateau or giving us a datapoint where we might see a little bit more robust response and give us some idea of whether we are seeing something.

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I don't know if C.K. knows whether the system can go above--

DR. CHOU: That is the other limitation by the engineering aspect. The higher the dose, of course, the similar gradient becomes more difficult, more difficult to control the temperature and also in terms of the generator. We had this--there was 1 kW there and we were able to do it. But some other labs, you don't have that kind of capability. It costs a lot of money to buy a high-power generator.

DR. OWEN: I will try another one like that. Likewise, further definition of the time course has been mentioned here and there. There may be some questions about which types of cells you are using and what the answer to the time-course questions would be.

There was also some mention of fractionated doses which is somehow related to time course as well. Can I get any other input on the topic of time course and what might be done or what questions there are to address? I would include in that the issues of when the exposure is with respect to the stages of conducting the assay.

DR. TICE: What you have just done is sort of factorially increased not the complexity but the number of experiments that are potentially possible. Basically, what one does is one starts with what data--we have positive

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data. Both Joe and we, in a sense, have data that replicates with a 24-hour exposure.

Joe was negative at 18. It was not even increased at 18, if I remember.

DR. ROTI ROTI: 16.

DR. TICE: So, in a sense, we have negative at 3 and positive at 24. So you titrate time. But you almost have to decide that time is an important variable, and we all think it is. And then, once you have titrated time where you know where you start getting increases, then you go back and do fractionation.

We do the same thing we did with the irradiation, just go back to those things. But, in terms of the number of experiments, I wouldn't want to sit down and partition that out right now without having more data based on where the dose response is.

DR. WILLIAMS: Do you think the system is robust enough in its current status to let you do many fractionation-type experiments?

DR. TICE: Michael was correct. We have had lymphocytes sitting at 37 degrees and then stimulated them to divide six days later. So the lymphocyte population--or you can hold 3T3 cells theoretically at a state of quiescence, at least for a while, under deprivation, or something.

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So I think there are ways to manipulate the cells to be able to handle that and then to go back to what Jim MacGregor was talking about is easy. You do stimulation before you put the cells in the exposure and let them divide under those circumstances.

So all those experiments are doable. All those experiments are actually fairly straightforward. It is just how much money do you have for how much time it is going to take to do all the various factorial responses you are trying to resolve.

DR. WILLIAMS: Certainly, you can do an estimate of power beforehand and say what the standard variation is between donors and everything and state what kind of statistical relevance you want and predict the number of--

DR. TICE: You just better hope that I stay in science a long time or I am going to run out of blood.

DR. MOROS: I just want to mention that, without further research and development, the RTL systems are pretty much limited, perhaps, to--if we are talking about being able to use the entire existing positions in each of the RTLs, it is probably around 3 and 4 W/kg. So we are limited in a high SAR. But what we have to offer is a large number of cells that can be exposed.

With further research and development, we could increase the SAR but that would be something that--

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DR. OWEN: You mean, with pursuing the shimming or something?

DR. MOROS: Yes. I actually have a proposal that I have been writing to optimize the RTL design for higher SAR, for higher, hopefully, more uniform SAR. But that is a significant engineering R&D project.

Yet, we will can offer a large number of cells at lower SARs.

DR. ROTI ROTI: For this particular assay, right now we have one position at the highest SAR and one RTL. So all the studies I showed you were done with just one flask being able to be irradiated at a time, and then another flask could be irradiated at 3. So we had the very sharp SAR gradient around that point. And that is at 5.

So if we wanted to push it to 10, we may have to have a limited number of flasks at that SAR. But if we are going to use the RTL to get up to a comparable dose, we need to push it to 10 in order for this to be feasible.

So your design--I don't know how much feasibility data you have, but it you have feasibility data that can show that we could conceivably achieve 10 W/kg, I think that would be important.

DR. MOROS: If you remember one of the slides that I showed at the end of my talk showed the ratio of the average SAR with shimming over the average SAR without

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shimming. You may remember that the maximum, which was about 15-fold, was around 1100 MHz.

I shouldn't be telling you this, but conceivably you can design a shim that would be able to move that peak down to 150 or 125. But that is the sort of research I am talking about. If I am able to do that, then that means that I don't even need new amplifiers. I can achieve 15 W/kg average with the system that I have now.

My worry would be, then, to take care of the temperature-control problem which is also part of the proposal that I am envisioning. But either system, you are not going to be able to go to 15 without considerable temperature-control engineering. 10, obviously, is possible.

I showed you the data but I didn't really show you the potential of what the data may be saying.

DR. OWEN: I just notice on my watch it is five until 5:00. People here around the table have transportation coming shortly. I just want to get sort of a five-minute warning for today's session. So collect your thoughts about what you want to say in the next five minutes and let them know because we will have to try and close on time.

DR. WILLIAMS: Do we have to go to the higher SAR? I thought I heard Dr. Chou say that going to SARs, we would

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experience a greater heterogeneity. Can that be eliminated at higher SARs, heterogeneity?

DR. HOOK: No; the heterogeneity would be the same.

DR. CHOU: You have to use the same system.

DR. HOOK: Right; we would use the same system. The gradient, the thermal gradient, would be greater.

DR. CHOU: If you use a higher SAR.

DR. HOOK: If you use a SAR, but the distribution of SARs would be the same.

DR. CHOU: When I studied a long time ago, I was studying muscle contraction. I first got a frog muscle. I couldn't do it because the temperature wiped me out. Any little bit of power, the contraction changes a lot because the perfusion--it is not enough to cool that bundle of muscles in there.

So I had to go this very thin diaphragm muscle from the rat. It is only ten cells thick. It is a transparent muscle. With that kind of a tissue, we were able to cool it because perfusion was fast enough. For the frog muscle, it is impossible.

The same thing here. Depending on what vessel you use and how you cool it, there is a thermodynamic problem; how do you cool something in the middle--depending on how

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much power you put in, how you cool it, how big the tissue sample is.

DR. HOOK: C.K., could we somehow design what we have in the TEM cell so that at 1 W/kg or 2.5 W/kg we were not controlling the temperature sufficient so we produced something--could we design something like that?

DR. CHOU: For example, that Petri dish, it can be very thin. Also very uniform. Also, the cooling is right at the bottom and you are blowing air right through that surface. So that one is easier. Compared to the test tube, it is a much easier question because you don't have the thermal gradient like the other one has. All the cells are at the bottom and right next to the surface. It is easier to cool.

So, unless you change the boundary conditions--otherwise everything will be the same.

DR. MOROS: What was the reason for going to the test tube instead of staying with that-

DR. CHOU: Because the biological procedure--this is a standard procedure in the lab, due to the GLP.

DR. MOROS: That cannot be changed to flasks, flasks which can be sealed?

DR. HOOK: That got us into numbers, samples we could handle and that sort of question. But my recollection is, although it is more uniform in the--I don't know if

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coupling is the right word--but we needed to put in less input power for a test tube to reach the same SARs in a dish.

DR. CHOU: That's true.

DR. HOOK: If you remember back to the Salmonella system, you had to go to something like 99 W input power or something.

DR. CHOU: Yes; that is why we still use the test tube. But because the dielectric constant is different. So there are different issues.

DR. HOOK: We need a system that is uniform. We need a system that we could use for each type of biological. It was complicated, but that's why.

DR. OWEN: It looks like everybody is about ready for today and it looks like it is just after 5:00, so I will close today's session and see you in the morning at half-past 8:00. Thank you.

[Whereupon, at 5:00 p.m., the meeting was recessed, to be resumed at 8:30 a.m., Wednesday, August 2, 2000.]

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